

TRANSDERMAL IONTOPHORESIS OF TERAZOSIN:  
AN EXPERIMENTAL APPROACH IN THE  
TREATMENT OF CAUSALGIA

CENTRE FOR NEWFOUNDLAND STUDIES

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TRANSDERMAL IONTOPHORESIS OF TERAZOSIN: AN EXPERIMENTAL  
APPROACH IN THE TREATMENT OF CAUSALGIA

by

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## ABSTRACT

In causalgia (a sympathetic mediated pain syndrome), pain appears to be mediated by the upregulation of  $\alpha_1$  adrenergic receptors on the primary afferents of the peripheral nerves.

Considering the limitations of the existing treatment, it is postulated that an  $\alpha_1$  adrenoreceptor antagonist, terazosin, when delivered locally at a site distal to the nerve injury by transdermal iontophoresis will produce pain relief. Iontophoresis is a noninvasive and a rate controlled drug delivery system.

A sensitive analytical technique for terazosin (TRZ) in plasma was developed using a cyanopropyl solid phase extraction technique and high performance liquid chromatography with fluorescence detection. Analysis of spiked plasma samples indicated no significant differences ( $p > 0.05$ ) in intra- and inter-day variations. The Solid Phase Extraction (SPE) procedure yielded a complete recovery of TRZ from plasma samples, enabling the detection of concentration as low as 0.05 ng/mL. The method was used to quantify TRZ from plasma after an intraperitoneal dose in a rat model.

Results from *in vitro* and *in vivo* experiments suggested that the iontophoretic delivery of TRZ was significantly higher than the passive delivery ( $p < 0.05$ , and TRZ degradation was prevented by the incorporation of a salt-bridge in the electrode assembly).

It is widely regarded that iontophoresis of small molecular weight ionic compounds have similar pharmacokinetic profile to those administered parenterally. This implies that iontophoresis overcomes the rate limiting barrier (the stratum corneum) and the drug appears in blood immediately following delivery. However, in our studies, peak plasma concentration is reached in approximately 1.5 hours after a ten minute iontophoresis, suggesting a drug depot formation in the skin. The nature (bound/free form) and the exact site of reservoir formation is not yet known. It may be possible to take therapeutic advantage of such a reservoir effect to establish skin depot that is slowly absorbed over time.

**Key Words:**causalgia, iontophoresis, solid phase extraction, terazosin, salt-bridge, depot formation.

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## ABBREVIATIONS

A	Ampere; strength of current
AC	Alternating Current
Ag <sup>+</sup>	silver ion
AgCl	chemical formula for silver chloride
AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of Variance
AUC	Area Under the Curve, a pharmacokinetic parameter
AUMC	Area Under Moment Curve, a pharmacokinetic parameter
av wt	average weight
CA	California
CAS	Chemical Abstract Services
CH <sub>3</sub> CN	chemical formula of acetonitrile
cm <sup>2</sup>	square centimeter
C <sub>max</sub>	maximum plasma concentration at time t <sub>max</sub> , a pharmacokinetic parameter
CN	Cyanopropyl functional group
Co	Company
Cl <sup>-</sup>	chloride ion
CV	Coefficient of Variation
DC	Direct Current
e <sup>-</sup>	electron
eg	example (s)
FDA	Food and Drug Administration of USA
g	gram (s)
G	Gauge of the needle
H <sup>+</sup>	hydrogen ion
H <sub>2</sub> O	chemical formula of water
HIV	Human Immunodeficiency Virus
HPLC	High Performance /Pressure Liquid Chromatography
hr	hour (s)
ie	that is
ip	intraperitoneal
iv	intravenous
k <sub>e</sub>	elimination constant, a pharmacokinetic parameter
Kg	Kilogram (s), 10 <sup>3</sup> gms
KHz	Kilohertz (s), 10 <sup>3</sup> Hz
LA	Los Angeles
M	Molar
mA	milliampere, 10 <sup>-3</sup> A
mg	milligram (s), 10 <sup>-3</sup> g

min	minute (s)
mL	milliliter (s), $10^{-3}$ L
mm	millimeter (s), $10^{-3}$ meter
mp	melting point
n	number (s)
NaCl	sodium chloride
ng	nanogram (s), $10^{-9}$ gram
NJ	New Jersey
nm	nanometer (s), $10^{-9}$ meter
O <sub>2</sub>	chemical formula of oxygen
OH <sup>-</sup>	hydroxyl ion
p	probability value, a statistical parameter
PG	Prostaglandin (s), a type of chemical mediator
pH	negative logarithm of hydrogen ion concentration
pK <sub>a</sub>	negative logarithm of dissociation constant
PHR/phr	peak height ratio
PRZ	Prazosin Hydrochloride
rpm	revolutions per minute
SD	Standard Deviation
SMP	Sympathetic Mediated Pain
SNO	Serial Number
R <sup>2</sup>	Coefficient of determination, a statistical parameter
SPD	Sprague Dawley, a strain of rat
SPE	Solid Phase Extraction
St	Saint as in St. John
temp	temperature in degrees kelvin unless otherwise stated
TENS	Transelectrical Nerve Stimulation,
THF	Tetrahydrofuran
TI	Transdermal Iontophoresis
TRZ	Terazosin Hydrochloride
t-test	a statistical method of analysis
μA	microampere (s), a unit of current $10^{-6}$ ampere
USA	United States of America
USP	United States Pharmacopeia XXI, 1985, and supplements 1 to 9.
UV	Ultra-Violet
Vd	Volume of distribution, a pharmacokinetic parameter
wt	weight
w/v	weight in volume
v/v	volume in volume
5-HT	5-hydroxytryptamine, a type of chemical mediator

°C	degree celsius
γ	gamma, a Greek letter; a type of primary afferent; also a type of a polymorph
%	percentage
μg	microgram (s)
α	alpha, a Greek letter; types of adrenergic receptor; a type of primary afferent; also a type of a polymorph
β	beta, a Greek letter; types of adrenergic receptor; a type of primary afferent; also a type of polymorph
μm	micrometer, 10 <sup>-6</sup> m
0700hr	7'0clock in the morning
x	an English alphabet; also multiplication
+	plus; in addition to
←	an arrow; also leads to
>	greater than
<	smaller than



## DEFINITIONS

Allodynia:	Pain due to a stimulus that normally does not provoke pain (Mersky, 1986).
Causalgia:	A syndrome of sustained burning pain, allodynia, and hyperpathia after a traumatic nerve lesion, often combined with vasomotor and sudomotor dysfunction and later trophic changes (Mersky, 1986).
Hyperalgesia:	An increased response to a stimulus that is normally painful (Mersky, 1986).
Hyperpathia:	A painful syndrome, characterized by increased reaction to a stimulus, especially a repetitive stimulus, as well as an increased threshold (Mersky, 1986).
Pain:	An unpleasant sensory and emotional experience associated with usual potential tissue damage, or described in terms of such damage (Mersky, 1986).
Response Surface Method:	This method employs a factorial design that determines the optimum conditions.
TENS:	This method involves the production and transmission of electrical energy from the surface of skin to the nervous system by using a electrode near the site of injury.

## 1.0 GENERAL INTRODUCTION

### 1.1 Statement of Research Problem

Causalgia is a painful syndrome arising from partial nerve injury caused by a high velocity projectile (e.g., a bullet wound) striking any part of the peripheral limb. Pain appears distal to the site of injury (i.e., the hand or the foot) is usually severe and is often described as burning, stabbing and tearing in quality (Sato and Perl, 1991; Price et al., 1989). Characteristically, this is accompanied by allodynia, hyperalgesia, hyperpathia and can be aggravated by variety of emotional and physical stimuli (Bonica, 1990a). Unlike pain due to extensive nonneural tissue damage which often subsides within few weeks, causalgic pain persists for several months to years (Price et al., 1992).

In causalgia, the activity in sympathetic efferent neurons can exacerbate pain (McMohan, 1991). These patients are often said to have sympathetically maintained pain (SMP). The diagnosis of the disease is obscure and requires a complete patient history as well as physical and psychological evaluations of the patient (Roger and Valley, 1994). The diagnosis depends upon the symptoms (burning pain, allodynia and hyperpathia), signs (edema, sweating profile, temperature changes), objective measurements (e.g., skin temperature, radiographs, and triple phase bone scans) and clinical

improvement following sympathetic blockade (Roger and Valley, 1994) or phentolamine infusion (Raja et al., 1991).

In general, management of causalgia involves treating the precipitating injury (cleaning of wound) followed by sympathetic blockade (Hannington and Kiff, 1974) and aggressive physiotherapy (Bonica, 1990a). Intravenous regional sympathetic blockade with local anaesthetics or guanethidine (Nathan and Loh, 1978) produces prompt relief. The success of sympathetic blockade depends on: (1) the timing of the block in relation to the onset of pain; (2) the skill of the physician; and (3) the completeness of sympathetic block. However, the injection of sympathetic blocking agents is painful, the relief obtained lasts only a few days, and repeated blocks are required (Bonica, 1990a). Repeated injections not only produce local tissue damage, but also have potential side effects (Löofström and Cousin, 1988) including hypotension, nausea, vomiting, urinary retention and convulsions. When these methods fail, sympathectomy is carried out using either the chemical or surgical methods depending upon the condition of the patient. Surgical removal of the sympathetic ganglia in an otherwise healthy patients can produce long lasting relief, but it is not always successful. Treatment of emaciated patients with chemical sympathectomy produces only transient relief.

Other treatment strategies include transcutaneous nerve stimulation, direct neural peripheral nerve or dorsal column

stimulations. Relief of pain by these treatments was reported by Ordag et al. (1987), but large controlled studies are not available to assess their long term efficacies. Several adjunct agents such as antidepressants with analgesic properties independent of their mood altering properties (Kishore et al., 1990; Tura and Tura, 1990) and opioids (Becker et al., 1995) are beneficial. Based on the up regulation of  $\alpha$  receptors on the primary afferents of the injured peripheral nerves, oral adrenergic antagonists have been tried (Sato and Perl, 1991; Sato et al., 1993; Koltezenburg., 1989). Oral treatment with prazosin was effective in humans at a dose of 8 mg/day, but the systemic adverse effects such as orthostatic hypotension, dizziness, tachycardia, impotence, blurred vision (AHFS, 1996) and frequent drug administration limit (Abram and Lightfoot, 1981) its long term usefulness. Thus in humans, the need for a number of approaches to treat causalgia have been attempted with some success, but the vast majority of patients are inadequately controlled by the available therapy.

## **1.2 Specific Hypothesis of SMP.**

### **1.2.1 Adrenergic Receptors on the Primary Sensory Neurons**

Clinical evidence for the involvement of an  $\alpha$ -adrenergic mechanism in causalgia is evident from the pain relief obtained in humans following the infusion of phenoxybenzamine (Ghostine et al., 1984) or phentolamine (Raja et al., 1991)

and by the oral prazosin administration (Abram and Lightfoot, 1981).

Wall and Gutnick (1974) have noted that many myelinated and unmyelinated regenerating efferents in an animal model of neuropathic pain develop ongoing activity. This is dramatically increased by local administration of norepinephrine, or by stimulation of the lumbar sympathetic chain (Devor and Janig, 1981) and is blocked by  $\alpha$  adrenergic antagonists, but not by  $\beta$  adrenergic antagonists (Scadding, 1982). These results suggest that the damage nociceptive neurons triggers the expression of  $\alpha$ -adrenergic receptors (Sato and Perl, 1991; Sato et al., 1993; Koltezenburg., 1989; Treede et al., 1992; Campbell et al., 1988, 1992). It is believed that catecholamines, released from the sympathetic efferent fibers bind to these receptors producing depolarization. Repetitive activity sets off spike propagation along the sensory neurons to the spinal cord. This peripheral link between sympathetic efferents and sensory neurons can be enhanced and maintained by somatosympathetic reflexes, whereby nociceptive afferent input evokes local sympathetic efferent activity over multiple spinal segments (Beacham and Perl, 1964; Blinn et al., 1980). The sympathetic efferent activity stimulation enhances the sensitivity of the nociceptor by

causing vasoconstriction, ischemia, and alteration in vascular permeability. Sensitivity is further enhanced by the direct action of locally released substances like substance P, PGs, and bradykinins (Pierce et al., 1996).

Several patients treated with oral prazosin, an  $\alpha_1$ -antagonist showed improvement in pain, swelling and vasoconstriction (McLesky, 1983). Significant pain reduction was obtained with oral prazosin in a patient with recurrent foot pain, previously untreatable to surgical sympathectomy (Abram and Lightfoot, 1981). Oral and topical application of an  $\alpha_2$ -agonist, clonidine, was also effective presumably by inhibiting norepinephrine release (Glynn and Jones, 1990; Davis et al., 1990). However, the systemic effect of these drugs limit their long term use.

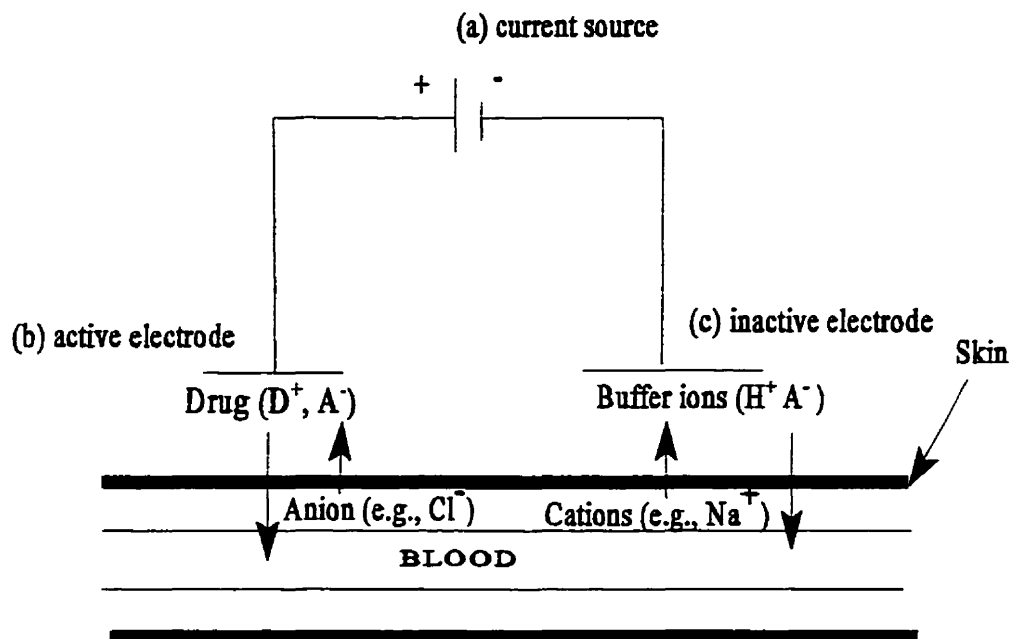
These results favour the involvement of  $\alpha_1$  receptors, and local delivery of an  $\alpha_1$  antagonist by transdermal iontophoresis may provide a convenient and effective method of pain control without producing predominant systemic actions. The dose of terazosin can easily be controlled by varying the strength of current and the time of delivery.

### 1.3 Introduction to Iontophoresis

The penetration of substances through the skin has long been used as the method for the administration of drugs to achieve a local, or a systemic action. Drugs such as scopolamine (Hyoscine®), nitroglycerin (Transdermal-Nitro®), estradiol (Estraderm®), and clonidine (Catapress®) are now commercially available as transdermal patches. However, some drugs when delivered passively ionize at skin pH, which result in a poor permeability across the skin (Sloan and Soltani, 1986). The number of solutes delivered systemically by transdermal iontophoresis is limited by the barrier properties of stratum corneum, the outermost layer. As a result, the conventional delivery systems are limited to highly potent, small lipophilic drug molecules.

Iontophoresis has the potential to overcome the limitations of conventional transdermal systems making it feasible to deliver ionic, hydrophilic and some of the high molecular weight compounds (Burnette, 1989; Sloan and Soltani, 1986).

Iontophoresis is a process whereby ionic molecules are transported into tissues by the passage of a direct electric current. The iontophoretic device (Figure 1.1) consists of three basic components: (a) a constant current source; (b) an



**Figure 1.1 A Typical Iontophoretic Delivery System:** The device shown can be easily mounted on the appendage such as the arm. It is assumed that under the electrodes exists an aqueous solution which on one side contains a positively charged drug ( $D^+$ ) and its counter ion ( $A^-$ ). The drug is assumed to act as its own buffer. Under the cathode exists a buffer which is designated in its dissociated form as  $H^+$  and  $A^-$ . Extracellular ions, primarily  $Na^+$  and  $Cl^-$  exist beneath the skin (Adapted from Burnette, 1989).



active reservoir containing the drug; and (c) an inactive reservoir. The drug in solution is delivered from an electrode of the same charge, and an oppositely charged electrode is placed at a neutral site (site where no therapeutic action is desired) on the body surface. When the device is activated, transport of charged molecules occurs across the skin primarily by electrostatic force of repulsion from the driving electrodes.

Thus, the migration of ions through the skin due to the applied electric field can be expressed as ionic current using Faraday's law of electrochemistry:

$$m = \frac{i_d \cdot t \cdot F}{Z} \dots\dots\dots \text{Equation (1)}$$

where:

$m$  = amount of drug delivered,  $i_d$  = strength of current carried by the drug ion,  $t$  = iontophoresis time,  $z$  = valency of drug ion,  $F$  = Faraday's constant.

The fraction of current carried by each ion is called the transference or the transport number for that ion. The maximum transport for a given ion occurs when the transport number is unity, i.e., when the ion carries 100% of current across the membrane. The transport number of drug also represents the efficiency of the iontophoretic system. Mathematically, the

relationship between the drug flux ion and the drug transport number is:

$$J_i = \frac{t_i \cdot I}{Z_i \cdot F} \dots\dots\dots \text{Equation (2)}$$

where:

$J_i$  = flux of drug ion,  $Z_i$  = drug charge,  $t_i$  = transport number for the drug ion =  $i_d/I$ ,  $i_d$  = current carried by drug ion,  $I$  = total current,  $F$  = Faraday's constant.

From the above equations, the efficiency of drug delivery will depend upon the extraneous ion concentration in the donor reservoir. The extraneous ion concentration can be reduced to maximize the iontophoretic efficiency (Sanderson et al., 1989). During iontophoresis, oxidation and reduction reactions may occur at the anode and the cathode. These reactions produce new ions that contaminate the drug solution. However, a careful selection of anode or cathode materials, in combination with the selection of an appropriate drug counter ion, could significantly reduce the electrochemical contamination (Coris et al., 1990). Using a weaker salt of the drug (Phipps and Gyory, 1992) (i.e., the acetate rather than hydrochloride salt) will improve the efficiency of drug delivery. The equilibrium between the weak anion and its corresponding acid will effectively prevent the pH in the

boundary layer from dropping below the  $pK_a$  of the acid. Using acetate as an example, the pH in the boundary layer (equal to its  $pK_a$  of -4.75) is sufficiently high to minimize proton transport, in spite of the high ionic mobility of protons.

Another modification is to increase the transport of drug ions at the expense of other ions by increasing the concentration of drug in the reservoir. This works to a point because, at higher concentrations the transport of the drug ion becomes independent of the drug concentration (Sanderson et al., 1989). Alternatively, double disc electrodes have been used to increase the morphine flux compared to the single electrode model (Clemessy et al., 1994). The use of chemical penetration enhancers such as hydro-alcoholic solution and surfactants (Srinivasan et al. 1990) may also increase drug permeation. There are several factors that affect the iontophoretic flux are described in section 1.4 to 1.6

#### **1.4 Physiochemical Factors Affecting Iontophoresis**

##### **1.4.1 pH of the Drug Solution**

The effect of vehicle pH on the rate and the extent of iontophoretic delivery of lidocaine through human stratum corneum was investigated by Siddiqui et al. (1985). The rate of penetration was greatest at the pH at which lidocaine

existed in the ionic form. The importance of pH in enhancing solute transport by iontophoresis was shown for other drug molecules such as insulin (Siddiqui et al., 1987a). A shift in pH becomes particularly important for protein and peptide drugs, since the pH of the solution determines the charge on these molecules (Siddiqui et al., 1987a, 1987b). Skin membrane ionization induced by pH change has been implicated to explain the anomalous behaviour of verapamil iontophoretic flux which increases with increasing the pH of the solution. The degree of drug ionization over the pH range was fairly constant (Behl et al., 1989).

#### 1.4.2 Molecular Size of Drug Ion

The permeability coefficient of a series of positively charged, negatively charged, and uncharged solutes across excised human skin was shown to depend on its molecular size (Yoshida and Roberts, 1992). As the molecular size increases, the permeability coefficient of the drug decreases (Yoshida and Roberts, 1993; Green et al., 1991). A drug ion with a molal volume of 150 mL/mol, based on the free volume model (Potts and Guy, 1991), is considered optimal for the iontophoretic transport (Yoshida and Roberts, 1993). However, certain solutes of higher molal volumes were shown to

penetrate effectively across the skin and into the systemic circulation. (e.g., insulin, vasopressin, and certain growth hormones)

#### **1.4.3 Ion Competition for Iontophoretic Transport**

The condition of electro-neutrality in solution requires that an equal quantity of positively charged and negatively charged molecules exist in a given volume of solution. Therefore, the migrating ion requires an ion of opposite charge in close proximity. This oppositely charge ion is referred to as the counter-ion. An ion of like charge, but of different type is termed a co-ion. Effect of these competitive ions on solute transport was shown by Bellantone *et al.* (1986) who observed the reduction in the benzoate flux to be more than half when an approximately equimolar amount of sodium chloride was added to the donor solution. Details of changes effecting ionic strength of the donor solution has been discussed by Lelawongs *et al.* (1989).

Use of divalent cations in drug solution is believed to diminish the selective permselectivity of skin towards cations by binding to negatively charged sites on the skin (Burnette and Ongipattanakul, 1987). The ionic composition of the receptor solution can also alter the efficiency of drug ion

transport. Replacing the competing ion ( $\text{Cl}^-$ ), with a polyacrylic acid yielded a transport efficiency of 80% (Phipps and Gyory, 1992).

## 1.5 Formulation Factors Affecting Iontophoresis

### 1.5.1 Current Strength and Types

A linear relationship is observed between the flux of most compounds and the applied current. The permeability coefficient of benzoate increased linearly when the current strength was increased from 0.08 to 1.6 mA/cm<sup>2</sup> (Bellantone et al., 1986). A linear increase in steady state flux with increasing current is reported for mannitol (Burnette and Ongipattanakul, 1987), thyrotropin releasing hormone (Burnette and Marreo, 1986) and verapamil (Wearly et al., 1989). Similar correlation was observed for 5-fluorouracil penetration in the rabbit eye (Kondo and Ariie, 1989).

Stephan and co-workers (1984) have pointed the necessity of using constant current, rather than constant voltage. Because at constant voltage, impedance of the skin changes rapidly during iontophoresis, primarily by the introduction of ions into the skin. The presence of these ions in skin increases skin conductance due to low skin impedance and high current. If constant voltage is used, current that passes

through the skin is highly variable. Given the fact, that at least a part of this current flows through the shunt (sweat gland and hair follicles) pathway, current densities can be quite high resulting in tissue damage. There are several types of current used in iontophoretic delivery (Figure 1.2). For small molecules such as lidocaine, at frequency up to 1 kHz, no difference in the delivery rate was reported for pulse or continuous DC mode of delivery (Bagniefski and Burnette, 1990). For large molecules such as insulin (Siddiqi *et al.*, 1987) and desmopressin (Nakakura *et al.*, 1996), pulsed current seemed to produce higher delivery. However, questions do arise about the design of pulsed DC power source operating at frequency above 10 kHz (Okabe *et al.*, 1986). At this frequency, electroporation of the drug can not be ruled out. Moreover, pulse DC system with 50% duty consumes twice the power of a continuous DC system. In most of the iontophoretic study constant DC current is used. But when used continuously over a period it produces skin polarization and irritation (Sanderson *et al.*, 1987). Hirvonen *et al.* (1995) have evaluated different current profiles that affect the iontophoretic delivery of charged aminoacids. In terms of total transmembrane drug delivery, constant DC was the most efficient, pulsed DC with a comparable charge did not deliver

as much as the uninterrupted DC, while square-wave and sine-wave AC profiles showed comparable transport rates.

Another novel method is the use of Pt/Pt electrodes with DC reversing current (Kara et al., 1993 ) (Figure 1.2). This mode of delivery has several advantages. First, the pH change in the donor and the receptor solution is negligible (Figure 1.3). Second, by reversing the electrode polarity, same drug or two different classes of drugs can be delivered simultaneously without physically mixing them. Third, the formation of vesicles and bullae that are basis for skin burns (Sanderson et al., 1987) are avoided by reversing the polarity of electrodes (Tapper, 1979). However, an obvious question that arises from this mode of delivery is: will reversing current remove the drug already delivered into the skin? This is unlikely, as reversing current will remove those ions (like  $\text{Na}^+$ ,  $\text{Cl}^-$  etc.) from skin that have a higher transport number than the drug ion.

#### **1.5.2 Electrode Types**

The choice of electrode is an important parameter for successful iontophoresis. If inert electrodes like platinum, carbon and graphite are used, electrolysis of water takes place at these electrodes, resulting in the formation of



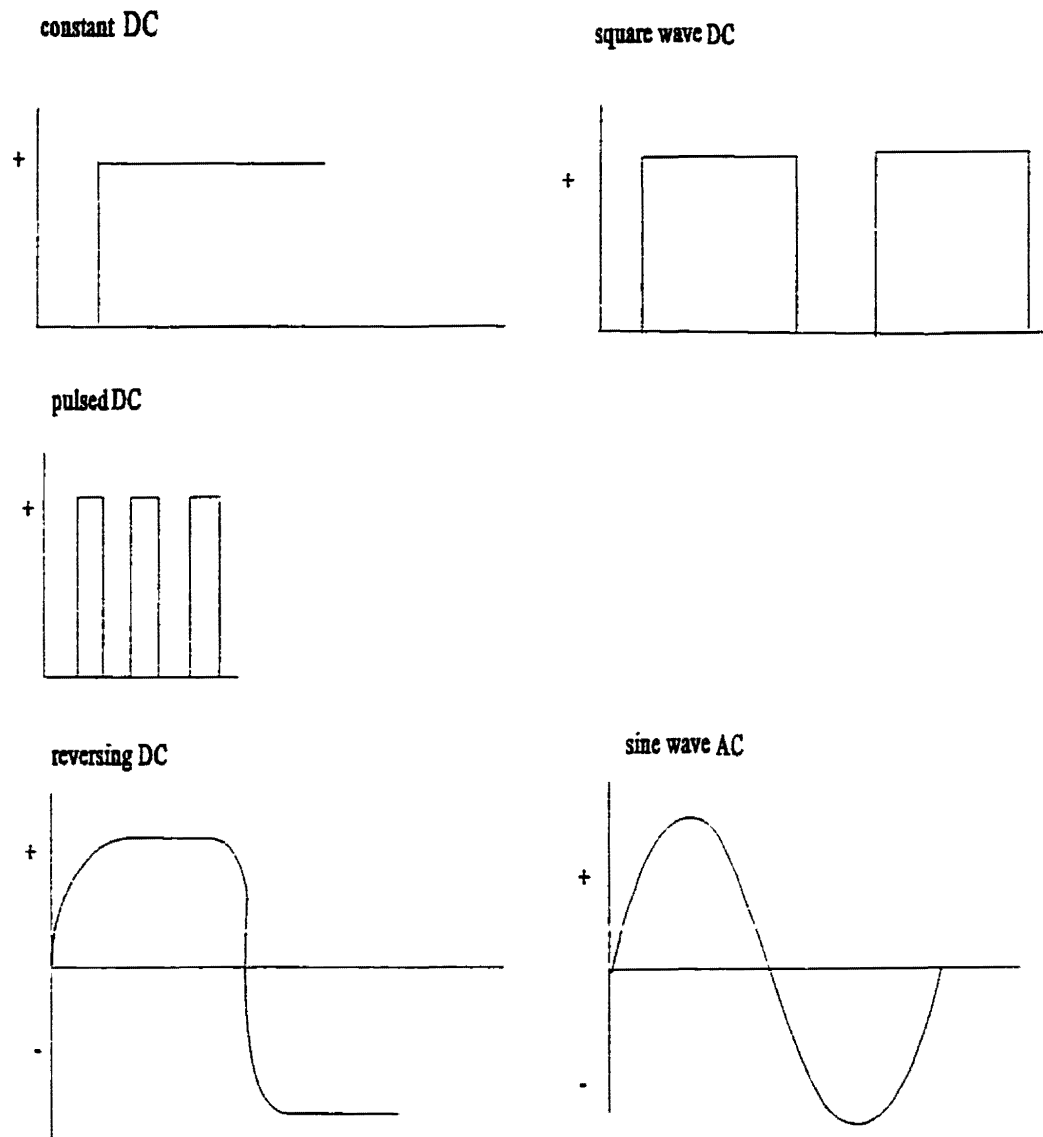


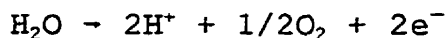
Figure 1.2 Types of Iontophoretic Current

hydrogen ions at anode and hydroxyl ions at cathode (Figure 3). Formation of these ions affects the pH of drug solution, and thereby affecting the drug flux (Sanderson et al., 1987). Moreover, the hydrogen ion being charged and highly mobile competes with drug ion for transport. The use of buffer does little to solve the problem. However, it does prevent the pH change, but provides its own ion for transport. Thus, decreasing the efficiency of drug delivery. These difficulties associated with electrolysis reactions that occur with inert electrodes in direct current (DC) iontophoresis can be corrected by using a size exclusion membrane (Burnette and Ongiattanakul, 1987). This membrane prevents direct drug solution interaction with the electrode. Replacing inert electrodes with Ag/AgCl that is consumed in the electrolysis (Figure 1.3) prevents the pH change. In addition, if drug molecules present in the system do not undergo redox reactions at Ag/AgCl electrode interface, the use of an Ag/AgCl electrode system provides a more convenient way to apply a voltage difference across the skin (if the total charge transfer across the electrodes during the experiment does not delete the AgCl deposited on the electrode).

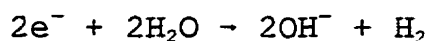
The Ag/AgCl electrodes are expensive and need constant replacement. Continuous use of Ag/AgCl electrodes may result

**Electrochemical Reactions:****(i) Inert Electrodes (e.g., platinum electrodes) and DC Current**

Anode (+)



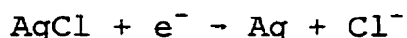
Cathode (-)

**(ii) Reactive Electrodes (e.g., silver/silver chloride) and DC Current**

Anode (+)



Cathode (-)

**(iii) Inert Electrodes with Reversing (every 5.0 min) DC Current**

Either Electrodes

$\text{H}^+$ (from anode reaction) +  $\text{OH}^-$  ( from cathode reaction)  $\rightarrow$  no pH change in the drug solution

**Figure 1.3 Electrochemical Reactions at Different Electrodes with Different Current Profiles.** A novel way is the use of DC reversing current, the  $\text{H}^+$  formed at the anode would neutralize the pH effect of  $\text{OH}^-$  ion formed at the same electrode (after the reversal of polarity). The pH change during the five minute interval is insignificant.

in deposition of black silver chloride over the gray Ag/AgCl layer as a result of chlorination. Mechanical removal of the deposit is generally not recommended. In such cases, a five to one dilution of dilute ammonium hydroxide with distilled water has been used with success (Boucsein, 1992). If a layer of bright silver appears, the electrodes can be rechlorinated, but the electrode layer is not uniformly composed of a sintered Ag/AgCl.

Given the significance of electrodes in iontophoresis with regard to drug delivery and patient comfort, an alternate way is the use of Pt/Pt electrodes and DC reversing current as mentioned earlier in the discussion (1.5.1).

## **1.6 Biological Factors**

The iontophoretic fluxes of pyridostigmine and lithium through human, pig and rabbit skin are comparable (Phipps et al., 1989). Iontophoresis reduces intra- and inter-subject variation in drug delivery rate, which is unachievable with passive absorption studies. This is consistent with the studies of Burnette and Ongiattanakul (1987) that observed comparable iontophoretic fluxes of sodium chloride and mannitol across human thigh skin obtained from the skin

regions of low and high density hair follicles. Insulin delivery from hairless rat skin was comparable to fuzzy rat (Siddiqui and Chien, 1987). Moderate enhancement of alcanoic acid iontophoretic permeation was observed in nude rat as compared to furry rat (DelTerzo et al., 1989).

Skin blood perfusion also influences drug flux. Epinephrine decreased the lidocaine flux due to vasoconstricting effects, and conversely, tolazoline increased the drug flux (Riviere et al., 1991; Riviere and Monteiro-Riviere, 1991).

### 1.7 Mechanism of Iontophoresis

The transport of a drug across stratum corneum may be transcellular (through the cells), or paracellular (around the cells), or by shunt pathway, or a combination of the above (Cullander, 1992). Accumulated clinical evidence suggests that the transport of ions occurs at least in part through a porous shunt pathway. This was demonstrated by the DC iontophoresis of a positively charged dye methylene blue, across excised human and mouse skin (Burnette and Marreo, 1986). The dye appeared on the excised skin in a dot like pattern and was observed on the receptor cell. Identification of the shunt routes for fluorescein dye was demonstrated with a pair of

scanning glass electrodes. When the micro-electrodes were moved across the localized dye, maximum voltage change was detected at the shunt region (Burnette and Ongpiattanakul, 1988).

In contrast, the transport of neutral molecules is not by direct electrostatic repulsion, but by current induced convective flow (Pikal and Shah, 1990a; Pikal and Shah, 1990b). Convective flow develops because the skin is negatively charged at physiological pH. As a result, the positive ions traverse through the skin to a greater extent than the negative ions. This sets up a net convective flow in the direction of positive ion transport. Thus, any solute that is present in convective flow will be transported across the skin.

The mechanism of iontophoretic delivery is complicated. Several mathematical models provide an insight into the factors governing iontophoresis. Applications of classical models have been reviewed by Burnette (1989) and Kasting and Keister (1989). A more recent membrane transport model is the Nerst Plank Diffusion Model (Kasting, 1992). The equation that describes this model has three components: a diffusion component\* an iontophoretic component\*\* and an electro-osmotic component\*\*\*. Symbolically, it can be written as:

$$J = Cu - D\{dc/dx\} + D\{zEFC/kT\} \dots \dots \dots \text{Equation (3)}$$

where:

$J$  = molar flux,  $C$  = molar concentration,  $u$  = convective water flow,  $D$  = diffusivity coefficient,  $dc/dx$  = molar concentration gradient,  $z$  = ionic valence,  $E$  = electric field,  $F$  = Faraday's constant,  $k$  = Boltzmann's constant,  $T$  = temperature.

The contribution of the convective flux to the overall transport is about 5% of the total iontophoretic flux for a solute with a transport number of about 0.2 (Yoshida and Roberts, 1992). The extent to which convective flux affects the transport of different solutes remains unclear. As this flow is directly dependent on the movement of the charged ions, convective flow will also increase with increasing current density (Burnette and Marreo, 1986). The theoretical analysis and initial experimental work by Pikal and Shah (1990a, 1990b) provide some additional insights. It is apparent from their analysis that; (1) the volume flow across the skin is independent of the induced osmotic effect; and (2) pore heterogeneity complicates the modelling of the volume flow effect, and its impact on the transport of different solute structures. In a separate study by Volpato et al. (1995) acyclovir delivery by electro-osmosis was found

significantly higher than the iontophoretic delivery.

### 1.8 Skin Effects

Passage of electric current through skin can provoke sensation ranging from tingling, itching, slight pricking to erythema. When lidocaine was delivered from a 4% aqueous solution of its hydrochloride salt over a range of currents ( $0.13\text{--}0.4\text{ A/cm}^2$ ) for period of 10, 40 and 100 mins, various degrees of erythema were observed (Monteiro-Riviera, 1990). Erythema in the drug-delivery-anode typically disappeared by 24 hrs and was minimum under the cathode (Monteiro-Riviera, 1990). A macromolecular capillary leakage as observed by FITC-dextran fluorescence, and histological demonstration of extravasated white blood cells are shown to be caused by DC current of 5, 20, and 50  $\mu\text{A}$ , or an AC of strength 20  $\mu\text{A}$  in hamster, chick and rabbit tibia (Nannmark et al., 1985). In a study by Rootman et al. (1988) iontophoresis (0.8 mA, 10 mins) in rabbit eyes was accompanied by reversible corneal epithelial edema with a small amount of mucosal discharge. Histological examination of the eyes showed circumscribed lesions. General irritation is the common side effect of iontophoresis (Ledger, 1992) and it is caused by electrical polarization due to continuous direct current. The possible



solution is the use of pulse or DC reverse current (Tapper, 1981). Electrical shocks experienced by some human volunteers were caused by high current density at the skin surface, which is avoided by increasing current strength gradually from zero to the desired value consistent with the patient compliance.

Tapper (1979, 1981) has suggested that the use of thick pad/gauge (>3 mm) beneath the negative electrode and the adherence to current-time limitation could help to avoid most of the iontophoretic burns (Tapper, 1979; (Ledger, 1992). Isolated cases of contact sensitization to ketoprofen and components of the electrodes were also reported by Teyssandier *et al.* (1977).

Despite the side effects of iontophoresis, the system when used properly can be extremely useful. Since parameters such as electrode-skin-contact area, current strength, the duration of current application (Prausnitz, 1996) and skin variation can affect the tolerability of a system (Ledger, 1992; Molitor and Fernandez, 1939), no *in vitro* methodology exists to assess these parameters. By experimental trials, a current strength of 0.5 mA can be safely applied to skin of electrode-skin contact area of less than 10 cm<sup>2</sup> (Sanderson *et al.*, 1989).

### **1.9 Optimization of the Iontophoretic Transport**

With respect to patient comfort, the iontophoretic transport can be regulated by varying the applied current density and the area of application. The pH and the ionic strength of the drug solution, and the current amplitude and frequency of pulsed current can be optimized by response surface method to ensure the maximum iontophoretic delivery (Huang et al., 1996a; 1996b). Also, an electrode of choice should prevent the pH drift during iontophoresis which otherwise may decrease the solubility of the compound. Selection of extraneous ions should be limited to ions with least mobility and conductivity. Constant reversing DC current or pulsed current should be used to minimize skin burns. Vasoactive chemicals (vasoconstrictors like epinephrine and vasodilators like tolazoline) may be used to facilitate either a local or a systemic delivery of ionic compounds.

### **1.10 Therapeutic Applications of Iontophoresis**

Iontophoresis of different classes of drug have been therapeutically used under different conditions: dentistry and oral conditions (Gangrosa et al., 1980), dermatology (Singh and Roberts, 1993; Glass et al., 1980) ophthalmology (Peggy et al., 1985; Grossaman and Lee, 1989; Rootmans et al., 1988),

otolaryngology (Rigano et al., 1992) and in miscellaneous conditions such as: hyperhidrosis (Holzle and Alberti, 1987); in the diagnosis of cystic fibrosis (Gibson et al., 1975); and intra-nasal dysfunctions (Weir, 1967). The iontophoresis of vinblastine in HIV-1 infected patients intolerant to systemic vinblastine in the management of cutaneous carcinoma widely improved the patient compliance (Smith et al., 1992).

### **1.11 Iontophoresis and Penetration Enhancers**

The use of chemical penetration enhancers with iontophoresis as to increase the transdermal delivery of high molecular weight polypeptides is suggested. Ethanol pretreatment of human skin was found to enhance insulin transport by a factor of 22 when compared to controls (Srinivasan et al., 1990). Use of epidermal enzymes such as phospholipase-C significantly enhanced the permeation of mannitol and testosterone across skin (Patil et al., 1996). These observations suggest that it may be possible to use penetration enhancers and achieve therapeutic levels at low current strength. Such a synergistic effect was also demonstrated for leuprolide and cholecystokinn-8-analogs (Srinivasan et al., 1990). However, the combination of iontophoresis and penetration enhancers did not increase the

permeability of Sotalol (Hirvonen et al., 1993). It is likely that the combination of iontophoresis and chemical penetration enhancer are effective in improving iontophoretic delivery of large molecular weight compounds as compared to small molecules. The use of proteolytic enzyme inhibitors such as camostat mesylate and aprotinin as absorption enhancers in iontophoretic delivery of peptides have been observed to facilitate the delivery of vasopressin (Morimoto et al., 1992a) and salmon calcitonin (Morimoto et al., 1992b) in rats.

#### 1.12 Iontophoretic Units/Device

Most of conventional iontophoretic system use a steady direct current in which the active electrode is incorporated in a moist pad or gauze in direct contact with the skin (Stillwell, 1971). In some cases, the indifferent electrode is larger than the active electrode and is placed at any convenient position on the body. This position does not significantly effect the iontophoretic flux (Lekas, 1979).

Three types of iontophoretic devices are commercially available: line operated devices (used in the device to diagnose cystic fibrosis), simple battery operated units (Phoresor® from Motion Controls Utah; Drionic® an FDA approved device from General Medical Co LA) and rechargeable power

sources (Hidrex® from Gesellschaft für Medizin and Technik, Wuppertal, Germany) (Tyle and Kari, 1988).

### **1.13 Limitation of Iontophoresis**

The iontophoretic delivery device is costly because of the electrodes, the electronic circuit, and the number of batteries required to sustain a therapeutic delivery rate. The possibility of unwanted effects of iontophoresis and drug on skin have been mentioned in literature but not adequately researched.

#### 1.14 Rationale and Specific Research Objectives

One way of treating causalgia is to interfere with the activity of sympathetic efferents that activate sensory afferents in the periphery. Alternately, beneficial effects might be expected if one could block the alteration in the central processing that leads to the abnormal pattern of the sympathetic outflow. Unfortunately, the nature of central processing is only now receiving more attention, and presently little information is available in literature as to its mechanism.

Whereas, the intrathecal administration of adrenergic agonists such as epinephrine produce antinociception in a rat model of causalgia, intravenous phentolamine (non-specific alpha adrenoreceptor antagonist) produces clinical pain relief in humans (Raja et al., 1991). These results indicate that  $\alpha$  adrenergic receptors are located in the periphery and not in the spinal cord. Also, these results are consistent with the human studies (Campbell et al., 1988, 1992, Treede et al., 1992, Abram and Lightfoot, 1992), suggesting that the main abnormality in causalgia is in the expression of  $\alpha_1$  receptors on the primary afferent fibers.

The present administration of alpha adrenoreceptor antagonists involve the invasive routes. This may result in

infection, damage to the wound, and even trauma. Since, iontophoresis is a noninvasive drug delivery system it reduces these unwanted effects. Iontophoresis shares the advantages of transdermal delivery, which include the bypass of hepatic first pass effect, gastrointestinal vagaries and controlled plasma level of potent drugs. The peaks and troughs associated with conventional dosage forms can be accurately controlled and titrated over time by employing iontophoresis. The inter- and intra subject variability is considerably reduced as the rate of delivery is proportional to the applied current (DelTerzo, 1989).

By definition, an  $\alpha_1$  adrenergic receptor antagonist when delivered distal to the site of injury in the appropriate dose (obtained from dose response curve) by transdermal iontophoresis will provide pain relief.

Controlled delivery can be achieved by altering the strength of the current, the duration of iontophoretic delivery, or both. Its advantage in treating a local condition like causalgia lies in its potential to deliver drug in high concentration to its site of action with minimal systemic uptake.

The ionic nature, high water solubility and  $\alpha_1$  receptor specificity make terazosin ideal for iontophoretic delivery in

the treatment of causalgia. To investigate this possibility, the following specific research objectives were undertaken:

- (1) To develop a sensitive method for the quantitative determination of terazosin in biological fluids.
- (2) To optimize the conditions for iontophoretic delivery and investigate the drug delivery in rat.



## 2.0 DEVELOPMENT OF SAMPLE PREPARATION TECHNIQUE FOR TERAZOSIN AND PRAZOSIN BY SOLID PHASE EXTRACTION AND HPLC.

### 2.1 Terazosin Hydrochloride (Martindale, 1993)

CAS: 63590-64-7b (terazosin); 63074-0(terazosin hydrochloride, anhydrous); 70024-40-7 (terazosin hydrochloride, dihydrate); 1-(4-amino-6,7,dimethoxy-2-quinazolinyl)-4-[(tetrahydro-2-furanyl)carbonyl-1] piperazine hydrochloride dihydrate  
Source: Abbot Labs, Montreal, Canada; Lot no 88615-AC-00

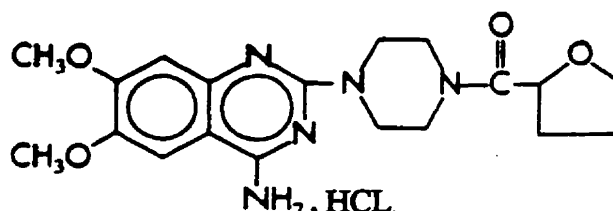


Figure 2.1 Chemical Structure of Terazosin Hydrochloride

$$(C_{19}H_{25}N_5O_4 \cdot HCL \cdot 2H_2O = 459.9)$$

#### 2.1.1 General Description

A white to off-white odourless powder, freely soluble in water and in methanol; mp, 272.6-274°C; pK<sub>a</sub>, 7.1 (proton gained). Terazosin exists as racemic mixture and both forms are pharmacologically active (Kyncl, 1986).

### 2.1.2 Pharmacokinetics, Metabolism and Stability (Titmarsh and Monk, 1987)

Terazosin is a specific  $\alpha_1$  receptor blocker and is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration (bioavailability is 90%). The drug is metabolized in liver and one of its metabolites has antihypertensive activity. Terazosin is 90 to 94% bound to plasma protein. It is chemically related to prazosin, but with a longer duration of action. After oral administration in rat, terazosin reaches  $C_{max}$  in about 3 hrs and has an elimination half life of 2.3 hours. In normal humans, the elimination half life is approximately 12 hours.

In solid state, TRZ is relatively stable under high thermal stress and photochemical degradation. However, TRZ rapidly degrades in a weakly acidic and basic solutions at elevated temperature (Chang and Bauer, 1991).

## 2.2 Prazosin Hydrochloride (Martindale, 1993)

CAS-19216-56-9 (prazosin); 19237-4-4 (prazosin hydrochloride), 1-(4-amino,6,7-dimethoxy-2-quinzolinyl)-4-2-furoyl) piperazine monohydrochloride

Source: Sigma Chemicals, St Louis, MO, USA; Lot no 13H0197.

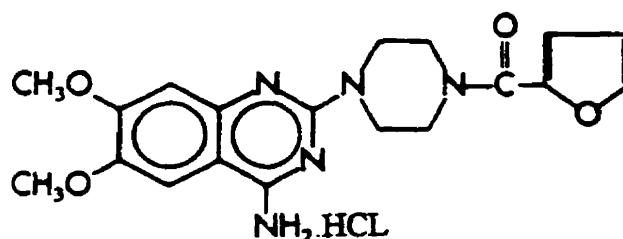


Figure 2.2 Chemical Structure of Prazosin Hydrochloride

$$(C_{19}H_{21}N_5O_4 \cdot HCL = 419.9)$$

### 2.2.1 General Description (Koster, 1989)

A white to tan colored, odorless or almost odorless powder. Very slightly soluble in water and in alcohol, slightly soluble in methyl alcohol and in dimethylformamide; practically insoluble in chloroform and acetone. Prazosin exists in various polymorph ( $\alpha, \beta, \gamma$ ) forms. The alpha form can be reproducibly manufactured. It is superior to the other

polymorphs due to ease of handling, storage, stability and formulation. The alpha form is used clinically. The drug is sensitive to light and must be stored in amber colored vials.

#### 2.2.2 Pharmacokinetics, Metabolism and Stability (Koster, 1989)

Prazosin is readily absorbed from gastrointestinal tract. After oral absorption, the peak plasma concentration is reached in 1 to 3 hours. The oral bioavailability is variable, ranging from 43 to 58%. Prazosin is 92% bound to plasma proteins. Prazosin is extensively metabolized in liver and some metabolites have hypotensive activity. Excretion of the metabolites and unchanged prazosin is mainly through the feces. The elimination half life in normal humans is 3.9 hours.

#### 2.3 Development of the HPLC Technique for Terazosin

Previous methods of measurement of prazosin and terazosin in biological fluids are based on spectrophotometry (Wood et al., 1976) and high performance liquid chromatography with UV (Paterson, 1984) and fluorescence (Bhamra et al., 1986) detection. The HPLC analytical technique for TRZ used in our research was adapted from Bhamra et al. (1986). Due to delay

in elution time and peak tailing of TRZ and PRZ, the above method {column: Spherisorb S5W silica (25 x 0.5 cm); mobile phase: 10 mM of ammonium perchlorate in methanolic solution at a flow rate of 2.0 mL/min} was altered to improve the desired resolution and sensitivity.

NOVA-PAK® C<sub>18</sub> column was used. The mobile phase consisted of 0.1 M phosphate buffer (pH 7.0), CH<sub>3</sub>CN and THF in a volume ratios of 76:18:6. At a mobile phase flow rate of 1 mL/min, terazosin and prazosin showed good fluorescence. The peaks were sharp and completely resolved from one another. The standard elution time of TRZ and PRZ was  $3.2 \pm 0.3$  and  $5.8 \pm 0.5$  min respectively.

Detection of the compounds were done either by UV (245 nm) or by fluorescence method (ex 375 nm, em 400 nm). The assay sensitivity by the fluorescence method was 0.05 ng/mL as compared to 45 ng/mL for the UV method at a signal to a noise ratio of 6. Due to marked difference in the sensitivity of the two detection methods, the UV procedure was qualified to *in vitro* studies while the fluorescence method was primarily used for *in vivo* study. Additionally, fluorescence detection reduces analytical interferences from other plasma substances.

The traditional TRZ extraction technique from plasma involve liquid - liquid extraction (Paterson, 1984; Bhamra et

al., 1986). These methods require large sample size and involve lengthy extraction procedures. Alternatively, we developed a solid phase extraction (SPE) technique for TRZ and PRZ as described in the section 2.7 (Singh et al., 1996). The SPE procedure is preferred over traditional extraction techniques with regards to time, labor, efficiency of extraction, high drug recovery and assay reproducibility (Krishnan and Abraham, 1994). The high recovery enables the quantification of small amount of drug. Furthermore, the SPE technique uses relatively small amount of organic solvent making the process environmentally friendly. By integrating SPE with HPLC, complete automation of the sample analysis can be achieved.

#### 2.4 Introduction to Solid Phase Extraction (Blevin et al., 1993)

Solid phase extraction is a physical process of separation of drug from unwanted matrix involving a liquid and a solid phase. The principle of separation is similar to HPLC. In SPE, the solid phase has a preferential attraction to the solid sorbent than to the solvent in which the isolate is dissolved. As the sample solution passes through the sorbent bed, the isolate gets bound to the sorbent, while the unwanted

sample components pass through. A very selective extraction resulting in a highly purified concentrated isolate can be achieved by choosing a suitable sorbent. Retention of the isolate depends upon the physio-chemical properties of both the sorbent and the isolate.

Bonded silica has gained popularity as a sorbent due to its versatility and availability with a wide range of physio-chemical properties. Organosilanes (R) are attached to the silica substrate (-Si-O-) through a silyl ether linkage. The sorbent will have different physio-chemical properties depending on the functional group R. Bonded silica are stable at pH range of 2 to 7.5. Above pH 7.5, the silica substrate is susceptible to dissolution in aqueous solution. Below pH 2.0, the silyl ether linkage is labile and the functional groups on the surface will begin to cleave changing the sorptive properties nonreproducibly.

Solvation of sorbent is necessary for sorbent to interact reproducibly with isolates. In effect, solvation is the wetting of the sorbent creating an environment suitable for isolate retention. This is achieved by passing large volumes of polar solvents like methanol, acetonitrile, or isopropanol. Once solvated, the sorbent bed should not be allowed to dry before application of test sample. After passing the test

sample through the sorbent bed, the bed is drained to discard unwanted interfering substances. Subsequently, the sorbent is washed with a suitable solvent to selectively remove all other adhering interfering substances before the final elution of the analyte with an another solvent.

## **2.5 Selection of Solid Phase Extraction Columns**

There are two different approaches for extracting analyte from the test sample. In the first method, the sorbent has a higher affinity for the analyte to retain it, and at the same time allowing the unwanted components of the sample to pass through. In the second method, the SPE sorbent has a lower affinity for the analyte allowing it to pass, while retaining the unwanted components. In the former approach, the analyte is finally eluted with a suitable eluent, while in the latter no final elution is necessary.

The selective retention of the drug or the extraneous matter depends upon the nature of sorbent. It is also based upon the functional group present on the analyte and composition of the sample matrix. Each sorbent within a given extraction mechanism exhibit a unique property of retention and selectivity which may be quite specific for the isolate. If an extraction involves a nonpolar mechanism, it may be



necessary to test several sorbents within a given category to find optimum balance between high recovery and good clean up (little or no plasma contaminants in the eluant). For TRZ ( $pK_a = 7.1$ ), a hydrophilic and water soluble compound with an amino functional group, the cyanopropyl sorbent column due to its intermediate polarity proved a good choice. The interaction of CN-sorbent in a polar environment is due to hydrophobicity as well as the polar characteristics of silica and residual silanols (cr. Krishnan and Abraham, 1994).

## **2.6 Bond Elut®Columns (Varian Sample Preparation literature )**

Bond Elut® cyanopropyl cylindrical columns are made of polypropylene reservoir containing 50 mg to 10 g of sorbent. Smaller cartridges are useful when sample size is very small, or when very small volume is needed for maximizing drug concentration. The maximum drug retention capacity is about 5% of the sorbent mass.

## **2.7 Solid Phase Extraction of Terazosin and Prazosin**

### **2.7.1 Materials and Methods**

Prazosin was used as an internal standard for terazosin. Deionized water was obtained using Barnstead Nanopure II system (Barnstead, MA, USA). All other solvents and reagents were of HPLC and analytical grade. Human plasma from healthy volunteers was used for the preparation of calibration curve and validation of the analytical procedure. Cyanopropyl bonded silica SPE cartridges (Bond Elut®, 100 mg, 1mL) were obtained from Varian Sample Preparation Products, CA, USA.

### **2.7.2 Preparation of Standard Solutions**

Stock solutions of TRZ (equivalent to 0.4 mg/mL of terazosin base) was prepared in water and diluted to obtain five different standard solutions ranging from 10 to 320 ng/mL. Prazosin solution (equivalent to 5 µg/mL of prazosin base) was prepared in 10% methanolic solution for use as internal standard and stored in amber colored vials.

### **2.7.3 Sample Preparation and Extraction**

Plasma samples (0.5 mL each in five test tubes) were spiked with 50 µL of a standard solution of known TRZ concentration and 20 µL PRZ solution, as internal standard. A

sixth plasma sample (blank) contained only 50  $\mu\text{L}$  of water and 20  $\mu\text{L}$  PRZ solution. The samples were further diluted with 1 mL 0.1 M phosphate buffer (pH 7.0) and mixed with the aid of a vortex mixer. The samples were then allowed to stand while the SPE columns were being conditioned. The SPE columns were mounted on sample preparation manifold (Adsorbex®, E Merck, Darmstadt, FRG) and conditioned by treating sequentially, with 1 mL methanol, followed by 1 mL of buffer solution, taking care not to let the column go dry. Diluted plasma samples were added to the conditioned SPE columns and allowed to percolate slowly under reduced pressure. After air-drying the column for three minutes the columns were washed with two, 1 mL portions of buffer solution, with 1 minute intermittent drying. Then the analytes were extracted with two, 1 mL portions of extraction solution consisting of THF and  $\text{CH}_3\text{CN}$  in the respective volume ratio of 1:3. The eluate was evaporated to dryness with the aid of thermostated water bath (37 °C) and a stream of nitrogen gas. The residue was reconstituted in 200  $\mu\text{L}$  of mobile phase. Twenty  $\mu\text{L}$  of reconstituted sample was injected into the HPLC system. The PHR of TRZ to PRZ were measured and plotted against the spiked TRZ concentrations.

#### 2.7.4 Chromatographic System

The HPLC system comprised a Waters Model 510 solvent delivery system (Waters Associates, MA, USA), Shimadzu SIL 9A autoinjector and fluorescence detector (Shimadzu Corporation, Kyoto, Japan), set at an excitation wave length 345 nm and an emission wave length of 400 nm. The analytical column was a 5 x 10 mm cartridge packed with Nova-Pak® C<sub>18</sub> reverse phase particles (Waters Associates, MA, USA) fitted with a C<sub>18</sub> guard column. Responses were recorded as peaks of a recorder (Kipp & Zonzn, Delft, Holland) which was operated at speed of 10 mm/min. The composition of mobile phase was adapted from the work of Paterson (1984) and modified to suit our instrumentation set up. It consisted of 0.1 M phosphate buffer (pH 7.0), CH<sub>3</sub>CN and THF, in the respective volume ratio of 76:18:6 which provided the desired resolution and sensitivity of TRZ and PRZ. Mobile phase was clarified by filtering through 0.45 µm membrane filter (Millipore Corporation, Mass, USA), degassed by purging helium and was delivered isocratically (room temperature) at a flow rate of 1.0 mL/min.

#### 2.7.5 Method Precision

Precision of the analytical procedure was evaluated by determining the linearity of response over 10-320 ng/ml drug

concentration, assay detection limit, percentage drug recovery and procedure reproducibility.

#### **2.7.6 Linearity**

Standard calibration curve of PHR *versus* concentration of spiked standard solution of TRZ was assessed by the coefficient of determination obtained by linear regression analysis. The process was repeated on five separate occasions and the reproducibility was determined by measuring the coefficient of variation of the slopes of the curves.

#### **2.7.7 Reproducibility of Procedure**

Precision of the extraction method was determined by replicate analysis of plasma samples spiked with three concentrations (54, 100, 200 ng/mL) of TRZ. Multiple number of samples (half mL each) of these three test solutions were prepared on the same day and stored at -20 °C and analyzed periodically. Inter-day comparison was based on analyses, performed in duplicates, on five different days, over a period of fifteen days. Intra-day comparison was based on four determinations of the three test solutions performed on one day. Mean, coefficient of variations and F values for two-way ANOVA studies were calculated.

#### **2.7.8 Reuse of the SPE Columns**

Generally, the SPE columns are meant for single use and the manufacturer does not recommend its repeated use. However, we have explored the possibility of reusing the SPE columns for repeated use. The column was reconditioned as follows: immediately after extraction, 1 x 1 mL of methanol was passed through the cartridge, followed sequentially (with drying in between the steps) with 3 x 1 mL dichloromethane, 3 x 1 mL of water and finally, 1 x 1 mL of methanol. Till further use, the columns were stored in a closed container.

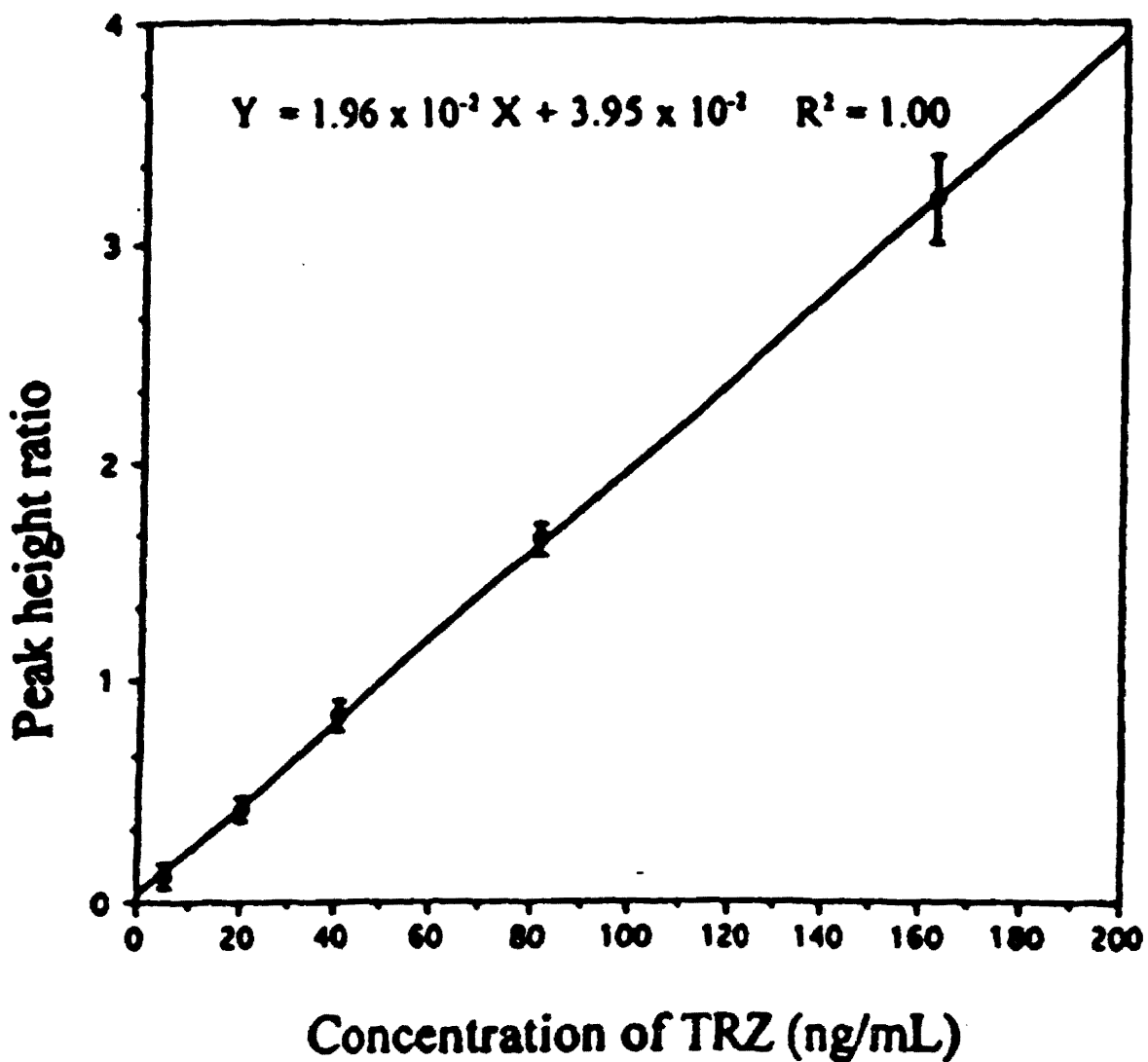
#### **2.7.9 Application to the Pharmacokinetics of TRZ in Rat**

The analytical procedure was used to study the pharmacokinetics of TRZ in rat. A male Sprague Dawley rat (Charles River, St Constant, Canada) weighing 473 g, was anaesthetized using 4% halothane and its jugular vein was cannulated with a 1 mm diameter polyethylene catheter, fitted with a three-way valve. This facilitated multiple blood sampling for drug analyses. Patency of the catheter was maintained with intermittent flushing with heparinized (10 units/mL) sodium chloride injection (0.9% NaCl, Astra Canada Inc, Mississauga, Canada). An intraperitoneal dose (0.33 mg/kg) of TRZ was injected. Blood samples (0.5 mL) were

removed via the catheter before the drug administration and at fixed time intervals over four hours during which period the rat was maintained in anaesthetized state using 1.5% halothane. Blood samples collected in heparinized polypropylene tubes were centrifuged immediately to obtain plasma which were stored at -20 °C until analysis. Before analysis, the plasma samples were allowed to thaw at room temperature and were extracted along with spiked standard TRZ in plasma and analyzed by the HPLC procedure described above.

#### **2.7.10 Data Analyses**

Calibration curves of PHR *versus* spiked concentration of TRZ were analyzed by linear regression analysis to obtain the equation of best fit. Inter-day variation in the analytical precision were assessed by using two-way ANOVA regression model with a significance level of  $p = 0.05$ . Intra-day comparisons were done by Student-t test and by calculating coefficient of variations. Concentration-time data following intra-peritoneal dose of TRZ was fitted with the help of a RSTRIP computer stripping program (Micromath Scientific Software, Utah, USA).



**Figure 2.3 Calibration Curve: Assay Validation.**

A plot of PHR versus concentration of spiked standard solutions of TRZ. Each point represents the mean  $\pm$  Sd of five determinations.



Table 2.1 Calibration Data: Assay Validation Studies (n = 5).

Conc. ng/mL	PHR d1	PHR d2	PHR d3	PHR d4	PHR d5
10.14	0.105	0.099	0.085	0.205	0.110
40.56	0.429	0.424	0.360	0.508	0.422
81.14	0.873	0.821	0.768	0.943	0.836
162.83	1.655	1.586	1.576	1.720	1.720
324.56	3.382	3.243	2.873	3.22	3.275

EQUATIONS OF CALIBRATION CURVES ON DIFFERENT WORKDAYS  $R^2$ 

1	PHR = 0.00207.CONC + 0.005299	1.00
2	PHR = 0.01980.CONC + 0.004267	1.00
3	PHR = 0.01782.CONC + 0.00298	9.99
4	PHR = 0.0191.CONC + 0.136	9.99
5	PHR = 0.0202,CONC + 0.230	9.99

CV SLOPE (m) (n = 5) = 5.79%

**Table 2.2 Assay Validation: Interday and Intraday Variation.**  
 Number of samples of each concentration (54, 100, 200 ng/mL) of TRZ were prepared and treated as unknowns.

Spiked amount of TRZ (ng/mL)		54	100	200
Inter-day variation (individual values, ng/mL))				
Day 1	52.22, 54.92	91.46, 96.46	190.18, 186.22	
Day 4	56.50, 53.94	98.44, 92.10	205.84, 200.72	
Day 7	56.02, 56.14	104.32, 104.26	204.88, 202.66	
Day 11	53.40, 54.76	98.00, 95.60	201.58, 201.46,	
Day 15	53.42, 53.86	98.80, 98.22	206.60, 197.12	
Mean.	54.52 ± 1.40	97.77 ± 4.28	199.73 ± 6.73	
± SD(n = 10)				
% CV (n = 10)	2.57	4.38	3.37	
Two-way ANOVA				
F value	0.22	0.22	7.07	
Intra-day variation (individual values, ng/mL)				
All samples analyzed	53.42, 53.86	98.80, 98.22	206.60, 197.12	
On day 15	52.62, 51.96	103.26, 99.40	210.40, 204.94	
Mean	52.97 ± 0.84	99.92 ± 2.28	204.76 ± 5.59	
± SD (n = 4)				
% CV (n=4)	1.59	2.29	2.73	
t-value	2.44	0.07	1.70	
Critical F-value (0.05, df, 4) = 7.71, Critical t-value (p = 0.025; df 3) = 3.1				

## 2.8 Result and Discussion

### 2.8.1 Linearity, Recovery, Detection Limit and Reproducibility

PHR obtained with human plasma samples spiked with the five different concentrations of TRZ over the concentration range of 10 to 324 ng/mL were linearly related to the spiked concentrations (Table 2.1). The equation of best fit for the line (average of five determinations) was:

$$Y = 1.96 \times 10^{-2} X + 3.95 \times 10^{-2}$$

where Y is the peak height ratio of TRZ peak to internal standard peak and X is the spiked TRZ concentration (Figure 2.3). Coefficient of determination obtained with the replicate determinations ranged from 99.7 -100 % and the coefficient of variation (CV) of the slope determined on the five separate occasions was 5.79 % (Table 2.1).

The SPE procedure was very efficient. The extraction procedure developed using cyanopropyl column selectively retained only TRZ and PRZ, leaving out minimum interfering substances as evident from negligible residue left in the test tube after evaporation of extraction fluid. The recovery of TRZ from plasma matrix by this procedure was virtually complete (range 100-104%) (Table 2.3). Complete extraction will enable one to quantify low drug concentrations from small test samples of 0.5 mL or less. The SPE procedure was also

**Table 2.3 Recovery ( $\eta$ ) of TRZ from plasma matrix** was determined by comparing the PHR of the extracted plasma samples containing known amount of analytes with those obtained by direct injection of standard solution without any treatment. The extraction efficiency is ratio of PES/PUS\* expressed in percentage.

Concentration (ng/mL)	Work Day	* $\eta\% = (\text{PER} / \text{PUS}) \times 100$  I $\eta\%$	*II $\eta\%$
10.14	1	0.1003/0.09786 = 102.5%	0.0853/0.0927 = 92%
	5	0.1002/0.09724 = 103%	
162.28	1	1.628/1.6443 = 101%	1.553/1.6421 = 94.5%
	5	1.557/1.5725 = 101%	

\*

$\eta\%$  = efficiency in percentage.

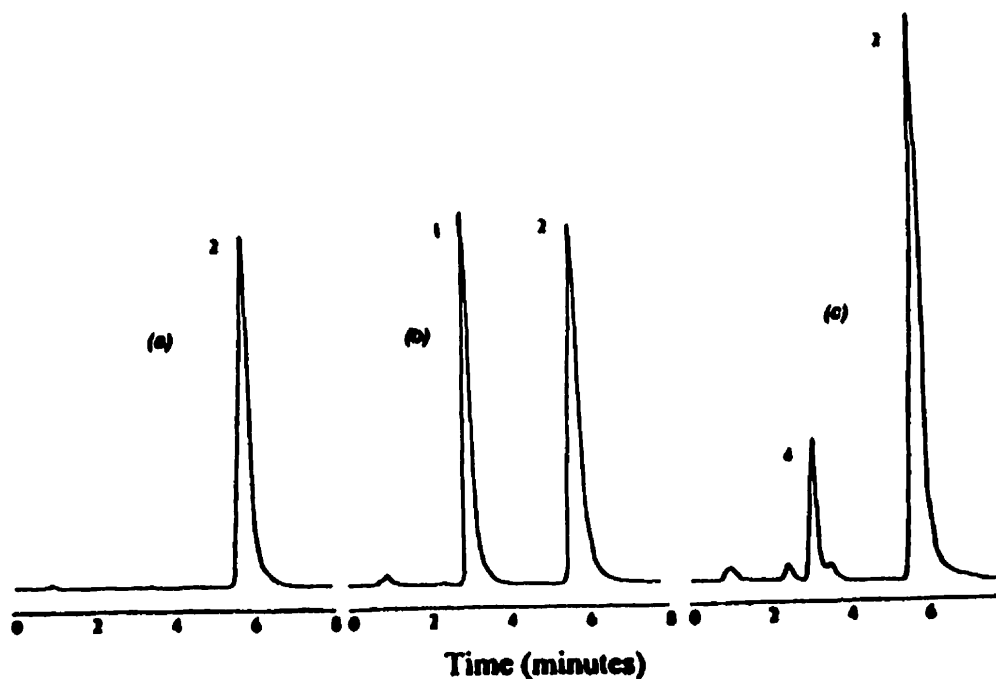
PES = peak height ratio (TRZ/PRZ) of extracted sample

PUS = peak height ratio (TRZ/PRZ) of unextracted sample

I $\eta$  = first extraction; II $\eta$  = second extraction

efficient with regard to time, labor and economy of organic solvent needed for extraction. With the use of a vacuum manifold 12 samples could be extracted in about 10 minutes. Only 2 mL of organic solvent was necessary for an extraction. Minimizing organic solvent waste made the SPE procedure environmentally friendly. The detection limit for TRZ in plasma was found to be 0.05 ng/mL, with a signal-to-noise ratio of 6 at an attenuation range 16 (i.e., the ratio of the output to recorder terminal was 16). This detection limit is an improvement over 0.25 ng/mL sensitivity reported by Peterson (1984). However, TRZ can easily be detected at a much lower concentration at three times the noise level.

The reproducibility of the analytical procedure was determined by both intra-day and inter-day variability studies. The % CV for the inter-day variability were 2.57, 4.38, and 3.37 respectively, for the spiked TRZ concentration of 54, 100 and 200 ng/mL (Table 2.2). Analysis of variance using two-way ANOVA regression model showed no significant difference ( $p > 0.05$ ) between determinations performed from day-to-day over 15 days. The intra-day %CV ( $n = 4$ ) for three test samples were 1.59, 2.28 and 2.73 respectively. Performance of t-test ( $p = 0.025$ ,  $df = 3$ ) on intra-day measurements showed no significant variations.



**Figure 2.4 TRZ and PRZ HPLC Chromatograms (assay validation)** : Figure (a) represents chromatogram of blank human plasma spiked with internal standard. Figure (b) represents the chromatogram of human plasma spiked with 5 ng of TRZ and 100 ng of PRZ. PRZ is structurally related to TRZ which makes it a good internal standard. The retention times of standard TRZ and PRZ were  $3.2 \pm 0.2$  ( $n > 100$ ) and  $5.7 \pm 0.4$  ( $n > 100$ ) minutes respectively. Figure (c) is the chromatogram of an inrat sample (spiked with internal standard) two hours after intra-peritoneal administration of 0.33 mg/kg of TRZ. The injection volume for all samples were same. Rat plasma sample (c) was analyzed at a higher sensitivity as to enable the accurate determination of peak height ratio.

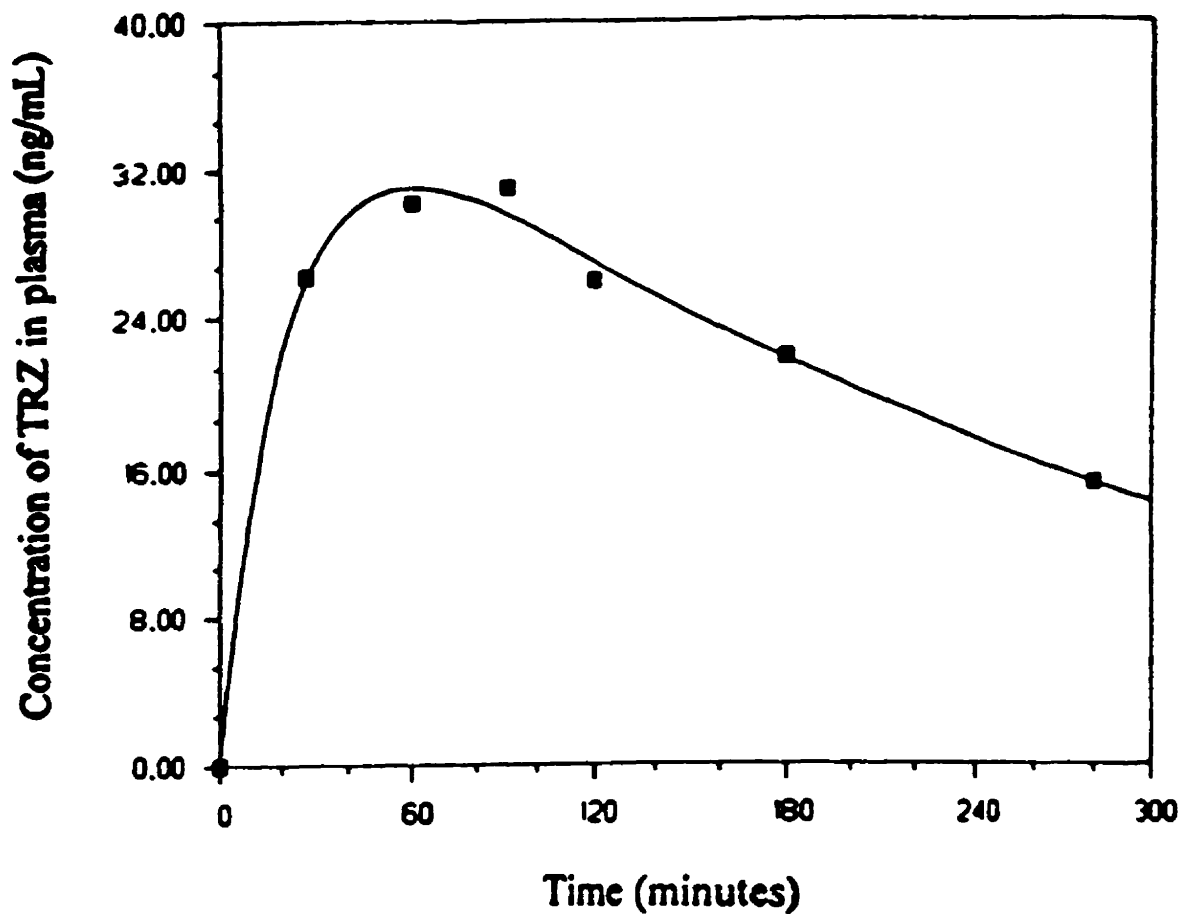


Figure 2.5 RSTRIP Simulation of TRZ Plasma Concentration-Time Profile After Single ip dose in a Rat. The values ■ shown are the experimentally determined concentrations after an ip dose of 0.33 mg/Kg of TRZ in a rat. The line drawn is the best fit line as determined by the RSTRIP program.

The results also indicate that TRZ in plasma stored at  $-20^{\circ}\text{C}$  remained stable over at least 15 days and all nonbiological samples were stable at  $5^{\circ}\text{C}$  for at least 3 months.

### 2.8.2 Pharmacokinetics

Terazosin plasma concentration-time profile after a single 0.33 mg/kg intra-peritoneal dose of TRZ is shown in Figure 2.5. The dose was selected based on literature data in the study of effects of TRZ in rats (Kyncl, 1986). The RSTRIP program chose biexponential fitting as the most suitable model and calculated certain pharmacokinetic parameters which are listed below:

elimination rates:  $k_1 = 0.0036 \text{ hr}^{-1}$ ,  $k_2 = 0.0466 \text{ hr}^{-1}$ ; Area under the concentration time curve  $(\text{AUC})_{0 \rightarrow \infty} = 10729 \text{ ng.min/mL}$   
 Peak concentration ( $C_{\text{max}} = 31.0 \text{ ng/mL}$ ) attained at 59.6 minutes ( $t_{\text{max}}$ ). Assuming complete absorption from the ip dose, the volume of distribution (1.17 L/Kg) was calculated from:

$V_d = (\text{AUMC} \times \text{Dose}) / (\text{AUC} \times \text{AUC}) \dots \dots \dots (\text{Gibaldi and Perrier, 1982}).$

Taking the slowest of the two rates as the elimination rate, the elimination half life was calculated to be 3.2 hours, compared to the reported half life of 2.3 hours after oral administration in rats (Chang and Bauer, 1992).



## 2.9 Conclusions

The SPE of TRZ from plasma and its HPLC quantification with fluorescence detection was found to be sensitive, precise, reproducible and efficient. The method can be easily adapted to monitor TRZ concentration in patients.

### 3.0 Transdermal Iontophoresis of Terazosin in Rats

#### 3.1 Introduction

Human skin consists of a highly vascular dermis, about 1000  $\mu\text{m}$  thick, and an avascular epidermis of about 100  $\mu\text{m}$  thickness. The epidermis can further be divided into three distinct layers consisting of the innermost basal layer, the intermediate granular layer, and the outermost layer the stratum corneum. It is generally agreed that the impermeable nature of the skin is primarily due to stratum corneum, which is about 10  $\mu\text{m}$  thick. Cullander (1992) has described stratum corneum as a protein rich corneocytes "bricks" surrounded by lipid rich "mortar." The intracellular mortar is composed of multicellular lipid bilayers. The lipid composition of the stratum corneum is unusual as it has large quantities of ceramides and free fatty acids. At normal body temperature these components are generally more ordered than other common membrane lipids. Therefore, the stratum corneum provides a greater barrier to the penetration of the substance than do other biological membrane such as the intestinal wall or the oral epithelium. Most of the lipids of the stratum corneum have order-disorder transition temperatures which are greater than the body temperature. In contrast, lipids of most cell membranes in the body generally have a transition temperature

less than the normal body temperature. Because of the unique structure and composition of the stratum corneum, lipophilic compounds tend to penetrate more readily than hydrophilic compounds in the presence of concentration gradient. However, under the influence of an electric field, ionic species can also penetrate the stratum corneum. Several investigators (Cullander, 1992; Burnette and Marreo, 1986) have identified hair follicles, sebaceous glands, and sweat glands as important routes (shunt pathways) of penetration for ionic compounds. Burnette and Marreo (1986) have shown that ionic dyes (eg., methylene blue) migrate through the skin via the pores identified as sweat and sebaceous glands. Using minute electrodes, Grimnes (1984) has found evidence of transappendageal migration of ions. While there is considerable evidence to support the ionic transport along the shunt pathway, migration of ionic species along the paracellular pathway cannot be ignored (Bodde et al., 1989; Lee et al., 1996). Accumulated literature fails to point out the exact transport pathway of ionic moiety. One of the several explanations is the enormous variation in skin samples in different animal species. Additionally, no other animal species other than humans possess the eccrine gland over the general skin surface. An animal skin homologous of human skin

that is completely appropriate for iontophoretic studies does not exist. The next choice is the human cadaver skin. Even that poses difficulties, especially in the availability of fresh samples.

Therefore, the bulk of research in this area depends upon the animal models. The animal model used are: hairless mouse (Durrheim et al., 1980), furry mouse (Behl and Barret, 1980), hairless rat (Garcia et al., 1980), nude rat (DelTerzo et al., 1986), fuzzy rat (Behl et al., 1985), hairless guinea pig (Friedlander et al., 1988), rhesus monkey (Wester and Maibach, 1976) and mini pig (Ainsworth, 1960). It has been found that in the passive diffusion studies, the hairless animals are far better models for simulating human skin than the furry animals (Behl et al., 1985). It should also be noted that hairless rats are not without hair follicles. They have some residual follicles in the skin and perhaps it is this feature that makes it a good model for transdermal study (Friedlander et al., 1988). Although some information is available about the nature of skin in passive drug transport, little is known about the animal skin for iontophoretic uptake. As evident from the contemporary literature both fuzzy and furry animals have been used (Siddiqui et al., 1987; DelTerzo et al., 1986). This lends support to the hypothesis

(Siddiqui and Chein, 1987; DelTerzo et al., 1989) that iontophoretic delivery is relatively independent of the source and the type of skin used.

In the proceeding sections, transdermal iontophoresis of TRZ in *in vitro* and in *in vivo* conditions are described. The following drug delivery issues have been examined: (a) stability of TRZ and *in vitro* delivery (b) drug delivery in rat. In the *in vitro* study excised fuzzy rat skin was used while in *in vivo* study both the furry and fuzzy rats were used.

### 3.2 Animals

All experiments were conducted using 250-300 g Sprague Dawley Rat and 250-320 g Fuzzy Rats (Charles River Canada, St Constant, Canada). Animals were housed in the Animal Care Facility, Memorial University of Newfoundland, at a room temperature of 22°C. A 12-hr light - dark cycle (light switched on at 0700 hr) was maintained, and the animals had free access to the rat chow and tap water. All experiments were conducted by the guidelines of the Canadian Council of Animal Care and were approved by the Memorial University Animal Care Committee.

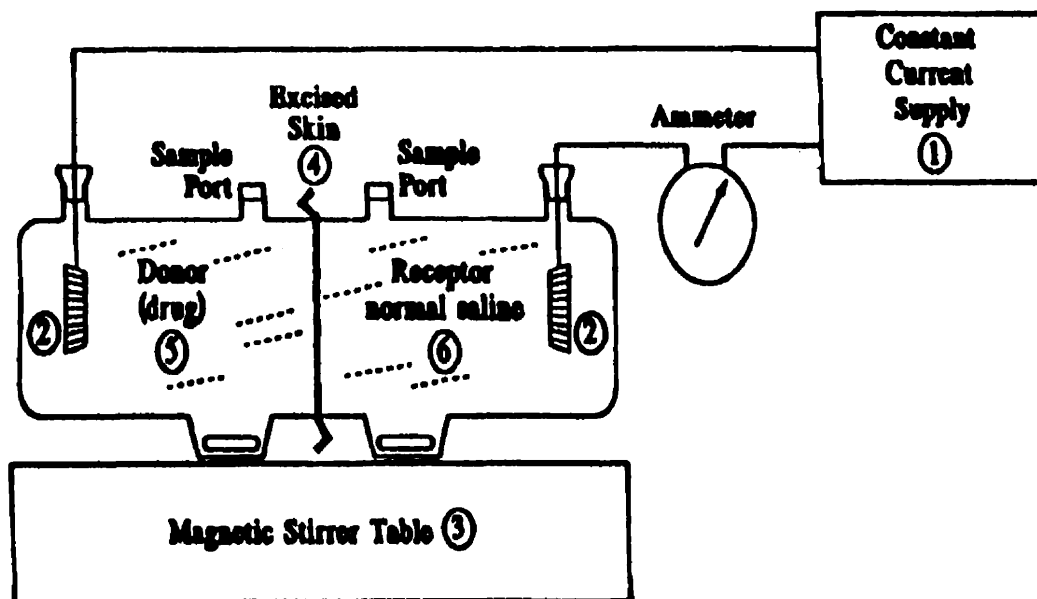
### 3.3 *In Vitro* Experiments

#### 3.3.1 Skin Preparation

Fuzzy rats were killed with an overdose of anesthetics (approximately 2 mL of 60% w/v urethane in saline). Skin from the abdomen region was surgically removed according to the procedure of Durrheim et al. (1980) and kept fresh over ice at 0-4°C (Swarbick et al., 1982) for a maximum of one week. Before use, a piece of skin of approximately 1.5 cm<sup>2</sup> surface area was cut from the excised skin and allowed to attain room temperature. It was then mounted on side - side diffusion cells (Figure 3.1) with the dermis facing the receptor cell.

#### 3.3.2 Experimental Set Up

*In vitro* experiments were carried out in diffusion cells. The experimental set up consists of following parts: a constant current supply (1) and two electrodes that convert the electronic current to ionic current (2). The donor cell contains the aqueous solution of the drug (5) and the receptor cell (6) contains the isotonic solution of sodium chloride. Magnetic stirrer (3) mixes the ionic moieties in the donor and the receptor cells. The cells were jacketed by circulating water and the temperature of the water was maintained at 37°C by a thermostat (Julabo Labortechnik, Germany). Fresh



**Figure 3.1** A Prototypical Glass Diffusion Cells. Volume of solution used is 3.0 mL. The electrode system was inserted into their respective ports and care was taken to avoid the formation of air gap adjacent to the skin.

exercised skin (4) was sandwiched between the donor and the receptor cells (drug permeation area =  $0.7 \text{ cm}^2$ ). After the electrode activation, sampling from the donor solution was done for every hour for the first 8 hrs and then after 24 hrs. Equivolume of solution removed was replaced by normal saline.

### 3.3.3 Stability of TRZ under Iontophoresis

Test run for the chemical stability of TRZ was investigated under 5 min reversing DC current ( $0.25 \text{ mA}$ ;  $0.7 \text{ cm}^2$ ) for 24 hours in glass diffusion cells using Pt/Pt electrodes. The drug solution ( $0.1\%$  w/v) was placed in both the donor (5) and the receptor compartments (6) but without the sandwiched skin. The electrodes were placed at the respective ports at  $5.5 \text{ cm}$  apart from each other. Samples were taken at regular time intervals and kept frozen at  $-20^\circ \text{C}$  till HPLC analysis.

During the iontophoretic run, the color of the solution changed from colorless to reddish brown. This suggested a possible drug degradation, which was confirmed by the HPLC analysis of the solution. The chromatograms (Figure 3.3 (i a)) exhibited an extra peak before the TRZ peak. Modification of platinum electrodes was done by incorporating a salt bridge in the electrode assembly (integrated electrode) (Figure 3.2).

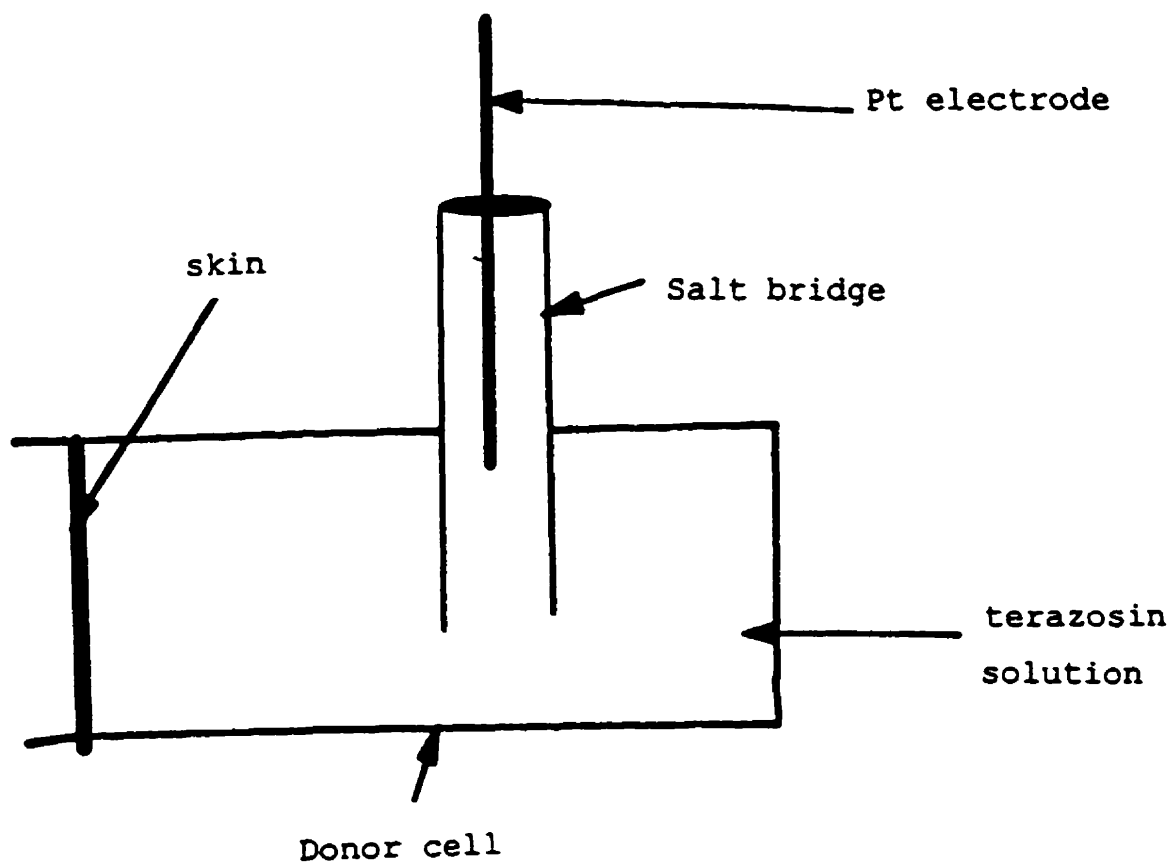


Repeating the above experiment with the integrated electrodes showed no color change in the drug solution. This was also confirmed from the HPLC analysis of the solution. The chromatograms exhibited no extra peak before the TRZ peak (Figure 3.3 (ib and iib)).

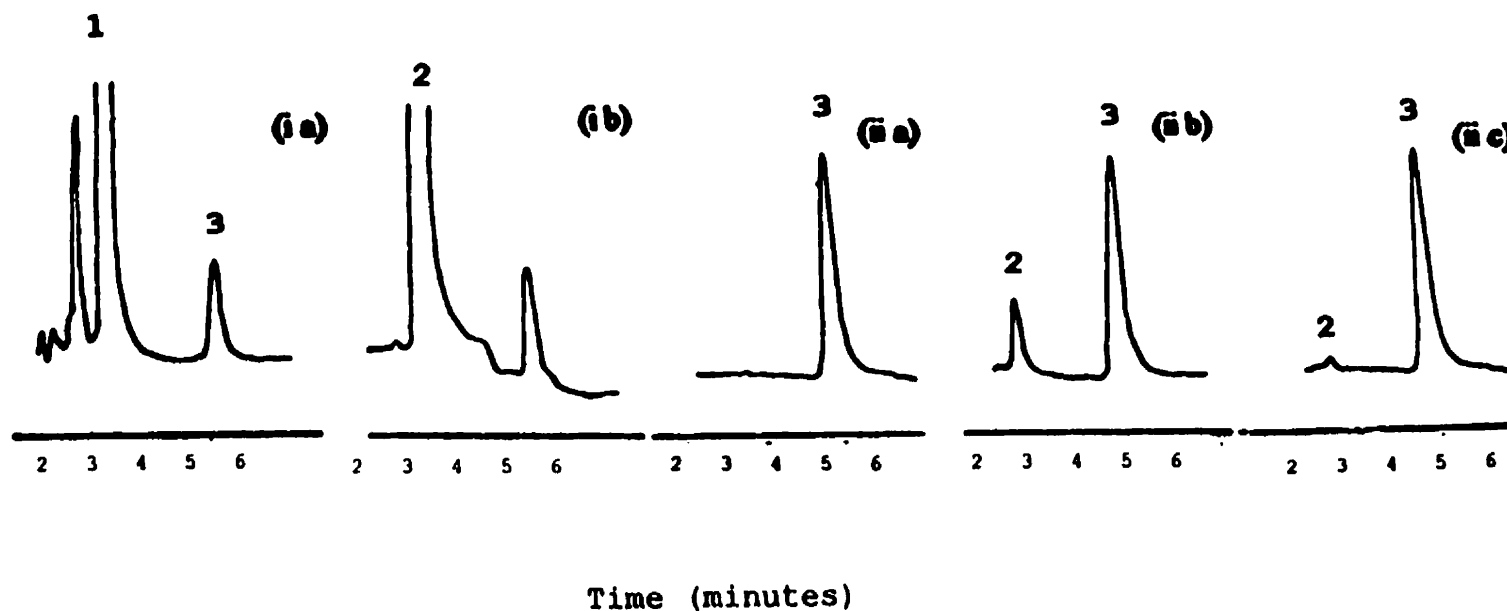
Although, the chromatograms of the coloured solution show a single additional peak before the TRZ peak, multiple peaks were also seen in UV analysis. It may be possible to identify and isolate these compounds in future experiments, but in the present context, no such study was carried out.

#### 3.3.4 Preparation of the Integrated Electrode

Agar grade-A (0.75 g) was dissolved in 25 mL of 0.9 % w/v sodium chloride solution and heated to boiling to give an agar-sodium-chloride gel. With the help of a 20 G<sub>1/2</sub> needle (Becton Dickinson, NJ, USA) fitted to a 5 mL syringe, the hot gel (temp 70-75°C) was filled into a 2.5 mm diameter 6 inches long polyethylene tubing and allowed to cool. During cooling care was taken not to allow the entrapment of air. The tubing were then cut into equal sizes of length 2.5 cm and wrapped in a parafilm (American Can Co, CT, USA) followed by aluminum foil. It was then stored at 0-4°C to prevent gel shrinkage and



**Figure 3.2 Assembly of the Integrated Electrode System:** Platinum electrode is inserted into one end of the salt-bridge to avoid a direct contact of the electrode with the drug solution. The potential drop across salt bridge was negligible.



**Figure 3.3 TRZ and PRZ HPLC Chromatograms (in vitro study).** Chromatograms (i a) shows an additional peak before the TRZ peak when current was passed through the drug solution using the Pt/Pt electrodes. However, no additional peak was noted when integrated electrodes were substituted for Pt/Pt electrodes (i b). Chromatogram (ii a) is from a blank sample; (ii b) is from the iontophoretically delivered TRZ sample (using integrated electrodes); and (ii c) is from a passively delivered TRZ sample. All samples were spiked with the internal standard (PRZ). The retention times of standard TRZ and PRH are  $3.1 \pm 0.2$  and  $5.6 \pm 0.4$  minutes respectively. Samples (i a) and (i b) were run at half sensitivity with respect to other samples

1 is a degraded compound peak; 2 is a TRZ peak; and 3 is a PRZ peak

possible microbial contamination. The tubings were used within 15 days of preparation. The electrode assembly is shown in Figure 3.2.

### 3.3.5 Sample Treatment

Frozen samples (500 $\mu$ L) from the receptor compartment were thawed to room temperature. Two samples of 100  $\mu$ L each were taken in 1 mL HPLC sample vials (Chromocol, Thrombill, USA). To each of these vial 22  $\mu$ L of PRZ solution (16.4  $\mu$ g/mL of PRZ in 10% methanolic solution was prepared as the internal standard) was added, and mixed using a vortex mixture (Thermolyn Corporation, Iowa, USA) for 30 seconds. Ten micro-liters of the sample solution was injected into the HPLC system. Detection was done by UV detector at 245 nm.

## 3.4 In Vivo Experiments

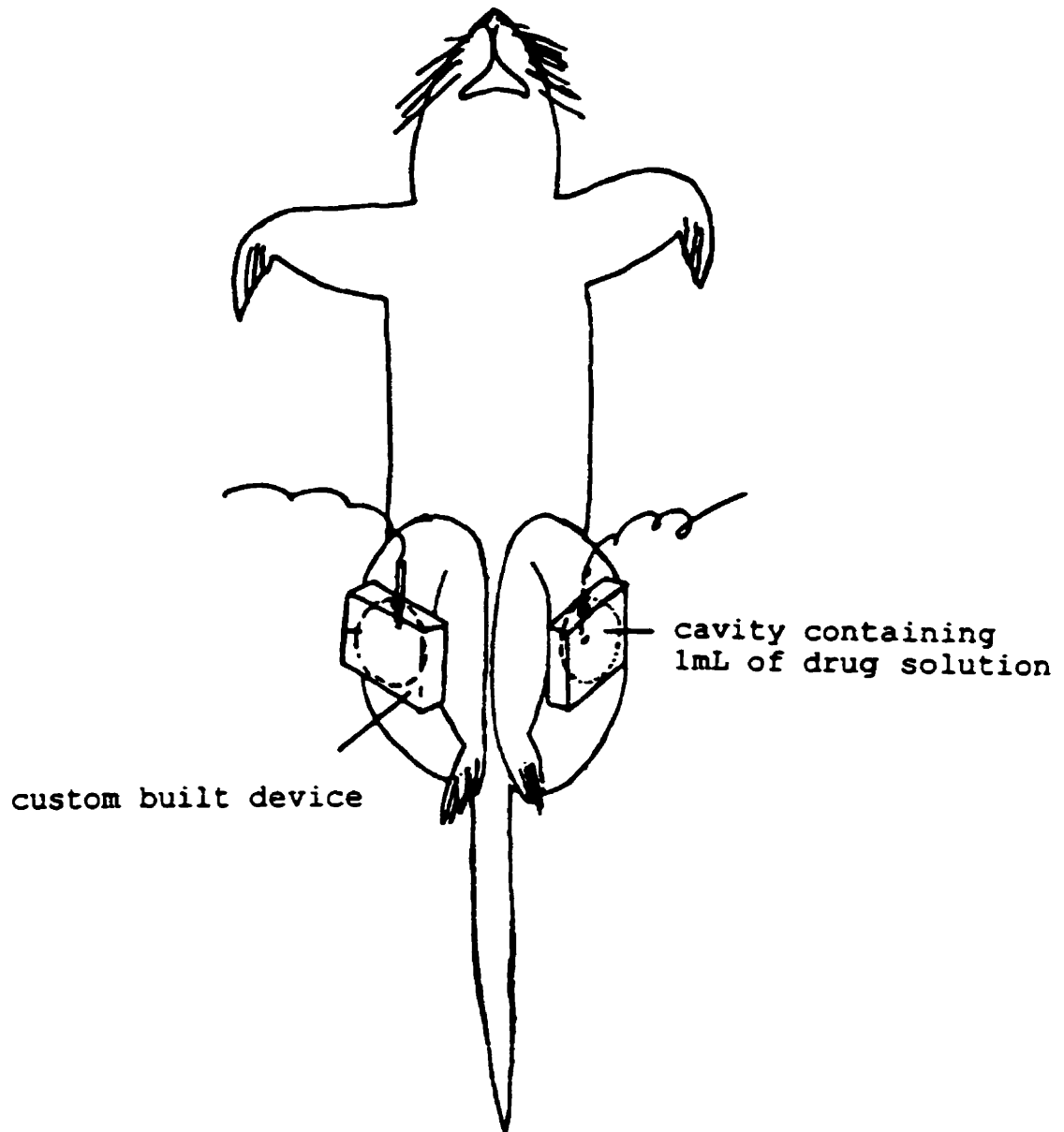
### 3.4.1 Animal Surgery

Male Sprague Dawley rats (Av wt 256 g, n = 7 (test = 5, control = 2)) and Fuzzy rat (275 g, n = 1) were anesthetized by an intraperitoneal dose of 20% w/v urethane in saline. Initial injectable dose was 1.1 mg/Kg body weight and when necessary, the dose was titrated upwards but not exceeding 1.5 mg/Kg body wt. Separate polypropylene catheters (1 mm

diameter) were inserted in the right carotid artery and left jugular vein. Microinjection pump (Carnegie Medicin, Stockholm, Sweden) was used to infuse 5 % w/v dextrose solution into the jugular vein at an initial rate of 5  $\mu$ L/min, and gradually increased and maintained at 22  $\mu$ L/min after 1 hour post drug delivery. Blood sampling was done via the right carotid artery. Patency of the catheter was maintained with intermittent flushing with heparinized (10 USP unit/mL) saline.

#### 3.4.2 Animal Preparation

Under anesthesia, body hair from a 2.5 cm<sup>2</sup> surface area of the upper left and right hind legs of the same rat were removed by a cordless mustache trimmer (Ronson®, Hongkong). Care was taken not to damage the skin. A pair of rectangular custom made plastic slabs (Figure 3.4) with a semi hemisphere cavity were glued to the shaved portion of the upper hind legs. The cavity was filled with 0.098% w/v TRZ solution. A single integrated electrode was inserted into each cavity on both hind legs through a small aperture provided at the top of the slab.



**Figure 3.4 Experimental Set Up: In Vivo Study.** The integrated electrodes were inserted into the cavity containing TRZ solution via the aperture at the top of the custom build device. The right carotid artery and the left jugular vein were cannulated for blood sampling and for dextrose infusion (not shown in the figure).

### 3.4.3 Drug Delivery

A power supply in combination with the electronic circuit (Kepco, NY., USA) delivered a constant DC current of 0.25 mA was used. When the device was switched on, the polarity of the integrated electrodes reversed every 5.0 min.

Initially, the drug delivery was done for 30 mins. But because of high mortality rate in rats due to cardiovascular effects associated with high plasma concentration of TRZ (50 ng/mL after 3.2 hr post delivery), the delivery time was decreased to 10 min.

On each of five different work days, separate iontophoretic runs were carried out for 10 min. After delivery, the plastic slabs were removed and the residual drug was wiped off the skin with a swab of wet cotton.

Blood samples (500  $\mu$ L) were collected at 0 hr, i.e., before delivery (blank) and 0.75, 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5 hr after delivery in a polypropylene tubes containing about 5  $\mu$ L of heparin (1000 USP unit/mL). Under identical conditions, the passive drug delivery experiment was done on two animals i.e., same experimental set up but no current was passed through the system. After the experiments the animals were killed with an overdose of anaesthesia.

#### 3.4.4 Sample Preparation and Treatment

Samples were centrifuged at 1000 rpm for 15 min. The plasma obtained was stored at -20 °C. Before analysis, plasma samples were allowed to thaw to room temperature. The plasma samples (150  $\mu$ L) were then spiked with 50  $\mu$ L of PRZ solution (3.2  $\mu$ g/mL of PRZ was prepared in 10 % methanolic solution and used as an internal standard) and treated to solid phase extraction procedure using cyanopropyl column as described in section 2.7.

Eluate obtained was evaporated to dryness with the aid of thermostat water bath maintained at 37°C and under a stream of nitrogen gas. The residue was reconstituted in 200  $\mu$ L mobile phases. Ten microliters of reconstituted sample was injected into the HPLC system.

#### 3.4.5 Chromatographic Conditions

The chromatographic conditions were identical to *in vitro* studies except the fluorescence detector (Shimadzu Corporation, Kyoto, Japan) set at an excitation and emission wavelength of 345 and 400 nm respectively was used instead of the UV detector.



### 3.5 Results and Discussion

#### 3.5.1 HPLC Assay Analyses

**Table 3.1 Calibration Data: In Vitro Study.** Interday variations were performed by analyzing following TRZ concentrations 0.084, 0.335 and 1.674  $\mu\text{g/mL}$ .

Conc.	phr	phr	phr	phr	phr	phr	phr	phr	mean phr
$\mu\text{g/mL}$	d1a	d1b	d2a	d2b	d3a	d3b	d4a	d4b	
0.084	0.127	0.125	0.137	0.121	0.127	0.130	0.128	0.133	0.128
0.164	0.200	0.204	0.201	0.212	0.192	0.212	0.210	0.225	0.207
0.335	0.374	0.396	0.365	0.335	0.373	0.378	0.424	0.422	0.383
0.837	0.894	0.926	0.887	0.808	0.928	0.919	0.987	0.984	0.916
1.674	1.850	1.920	1.640	1.600	1.920	1.923	1.951	1.984	1.848

#### EQUATIONS OF CALIBRATION CURVES ON DIFFERENT WORKDAYS

1a  $\text{PHR} = 1.084.\text{CONC} + 0.0178$ ; 1b  $\text{PHR} = 1.127.\text{CONC} + 0.0167$   
 2a  $\text{PHR} = 0.954.\text{CONC} + 0.5572$ ; 2b  $\text{PHR} = 0.922.\text{CONC} + 0.0484$   
 3c  $\text{PHR} = 1.136.\text{CONC} + 0.0058$ ; 3c  $\text{PHR} = 1.129.\text{CONC} + 0.0149$   
 4d  $\text{PHR} = 1.464.\text{CONC} + 0.0303$ ; 4d  $\text{PHR} = 1.612.\text{CONC} + 0.0312$

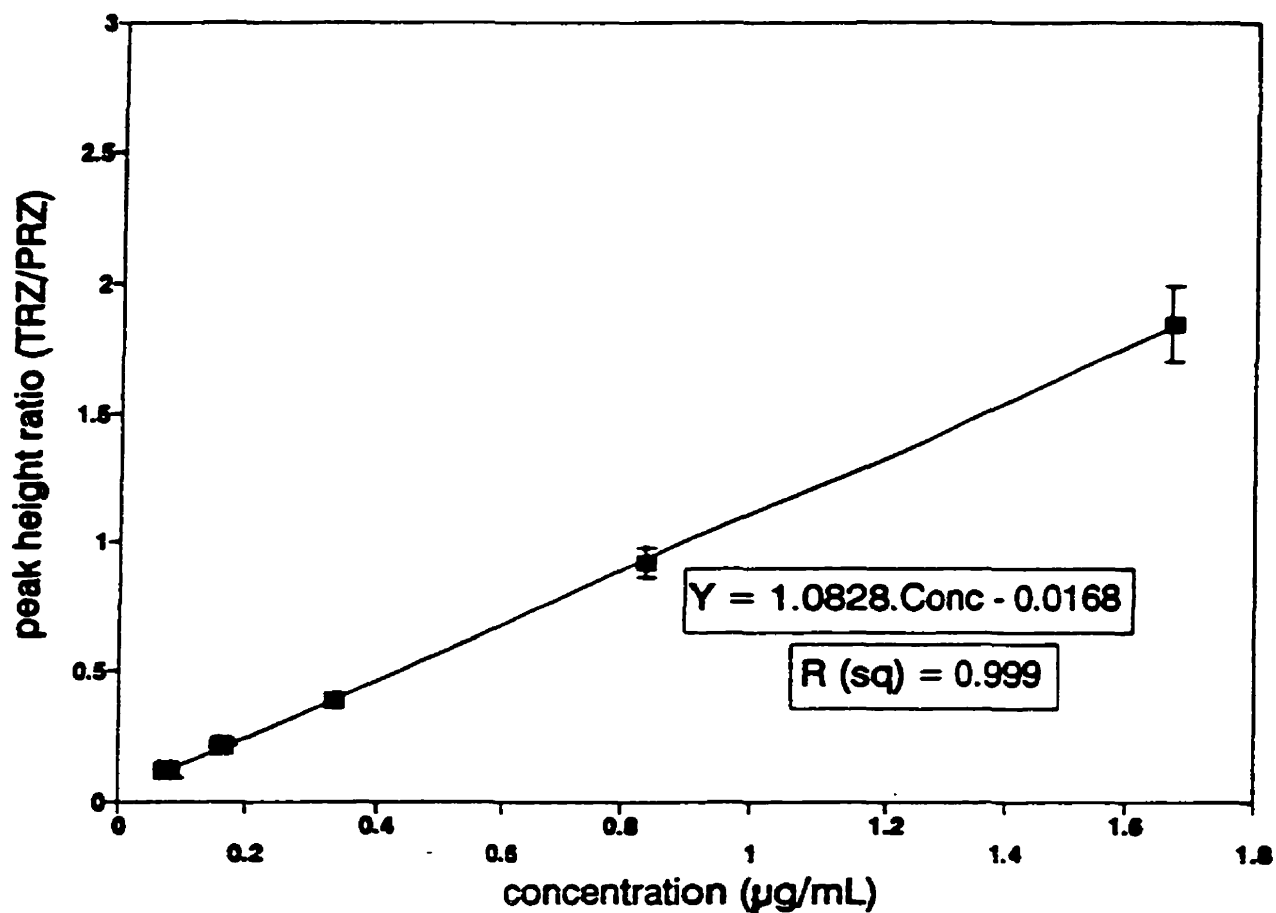
CV SLOPE (m) (n=8) = 10.98%

Two way ANOVA F values for the interday variation performed on the concentrations 0.084, 0.335 and 1.674  $\mu\text{g/mL}$  are:

0.97, 1.28, 1.85. Critical F values (df. 1,3 at 0.05) = 10.1

phr = peak height ratio of TRZ to PRZ; d1a = phr at the start of the day one;

d1b = phr at the end of the day one



**Figure 3.5 Calibration Curve: In Vitro Study.** A plot of PHR versus concentration of spiked standard solution of TRZ. Each point represents the mean  $\pm$  SD for 4 work days (■) (n = 8).

**Table 3.2 Calibration Data: In Vivo Study.** Precision of the analytical procedure determined by standard deviation and co-efficient of variation.

CONC ng/mL	PEAK HEIGHT RATIO (TRZ/PRZ)							MEAN ± SD	%CV
	day 1	day 2	day 3	day 4	day 5	day 6	day 7		
6.16	0.120	0.190	0.144	0.175	0.221	0.164	0.171	0.169 ± 0.03	17.9
12.32	0.244	0.280	0.289	0.318	0.302	0.288	0.298	0.289 ± 0.02	7.62
24.64	0.607	0.570	0.600	0.544	0.559	0.550	0.563	0.570 ± 0.02	3.89
61.6	1.550	1.487	1.583	1.555	1.300	1.246	1.456	1.495 ± 0.11	0.84
123.2	2.626	2.548	2.760	2.631	2.510	2.464	2.651	2.598 ± 0.09	3.53

WORK DAYS	EQUATIONS OF CALIBRATION CURVES	R <sup>2</sup>
1	PHR = 0.0216.CONC + 0.04355	98.9
2	PHR = 0.0226.CONC + 0.04621	99.4
3	PHR = 0.0207.CONC + 0.06191	99.2
4	PHR = 0.0213.CONC + 0.07121	99.1
5	PHR = 0.0197.CONC + 0.08063	99.9
6	PHR = 0.0196.CONC + 0.04793	99.7
7	PHR = 0.0214.CONC + 0.05290	99.7

CV SLOPE (m) (n=7) = 4.66%

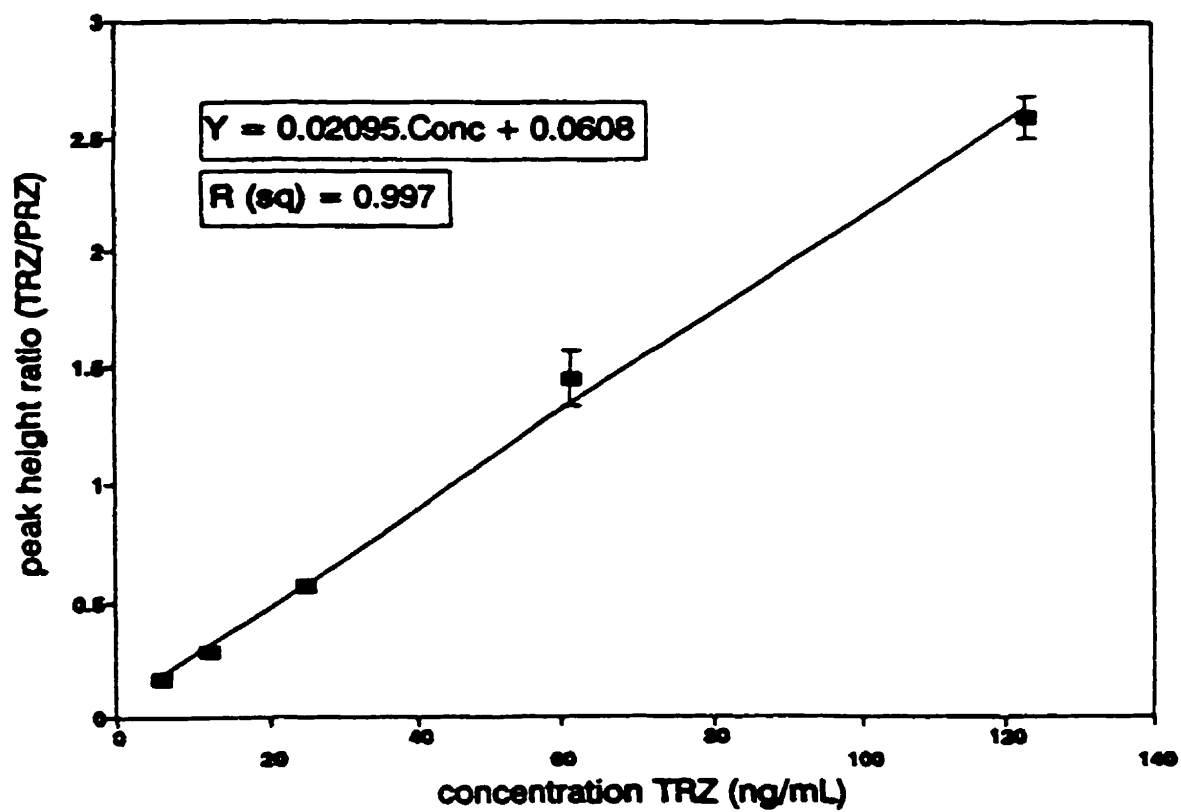


Figure 3.6 Calibration Curve: *In Vivo* Study. The values shown are mean  $\pm$  SD ( $n = 7$ ).

For *in vitro* studies, standard analysis (Table 3.1) of TRZ in the concentration range of 0.08  $\mu\text{g/mL}$  - 1.68  $\mu\text{g/mL}$  were constructed in duplicate for each of the five work days. The PHR of TRZ to PRZ was linearly related to the concentration of TRZ. Correlations of determination ( $R^2$ ) for the linearity for all standard curves were greater than 0.99. The reproducibility of the analytical procedure was determined by two way ANOVA. The between day and inter day variation were not significant ( $p > 0.05$ ). The coefficient of variation in slopes was less than 11% ( $n = 8$ ) (Table 3.1). The high value is probably due to analytical error in the standard sample (61.6 ng/mL).

For *in vivo* studies, the standard curves of TRZ in the concentration range of 6.6 - 123.2 ng/mL were constructed on each of the work day ( $n = 7$ ). The sensitivity of the assay procedure is 0.05 ng/ml at a signal to noise ratio of 6. However, the phr was easily determined at three times the noise level for the passive delivery (Table 3.3).

The PHR of TRZ to PRZ was linearly related to the concentration of TRZ ( $R^2 > 98.8$ ). Reproducibility of the analytical procedure was determined by co-efficient of variation of the slopes (less than 5% ( $n = 7$ )) (Table 3.2).

### 3.5.2 Pharmacokinetic Analysis

The elimination rate from the central compartment is  $0.0304 \text{ hr}^{-1}$ . The plasma half life is 2.25 hr and compares well with the existing literature value of 2.3 hr (Chang and Bauer, 1991). The clearance of the drug from systemic circulation is  $0.009 \text{ L/hr}$ . Assuming complete absorption, the area under curve  $((\text{AUC})_{0-\infty})$  is  $88.58 \text{ ng.hr/mL}$  was calculated from the trapezoidal rule. The residual area was determined from  $C_n/k_e$  where,  $C_n$  is the last observed plasma concentration. Since the volume of distribution is best estimated from iv dose, the estimation of iontophoretic dose and the transport efficiency are therefore approximate. (The volume of distribution is based on ip dose extrapolated from section 2.8.2.) It may be necessary to validate the results with iv dose determination.

$$V_d = \frac{(\text{AUMC}) \cdot \text{Dose}}{(\text{AUC})^2} \quad (\text{Gibaldi and Perrier, 1982})$$

$$\text{Dose} = \frac{(\text{AUC})^2 \cdot V_d}{(\text{AUMC})}$$

$$V_d = \text{av wt of rat} (0.256 \text{ Kg}) \times 1.17 \text{ L/Kg} = 300 \text{ mL}$$

$$\text{Dose} = \frac{(88.58)^2 \times 300 \text{ mL}}{279.11} = 8.43 \times 10^{-6} \text{ g}$$

If  $i_d$  is the strength of the current carried by the drug ion, then by Faraday's law:

$$i_d = \frac{\text{Dose. (Faraday's constant). (molecular charge)}}{(\text{molecular weight of terazosin}). (\text{time of application})}$$

$$i_d = \frac{8.43 \times 10^{-6} \times 96500 \times 1}{387.44 \times 600} = 3.5 \times 10^{-6} \text{ A}$$

$$\text{transport number } (t_r) = \frac{i_d}{\text{total current}} = \frac{3.5 \times 10^{-6} \text{ A}}{0.25 \times 10^{-3} \text{ A}}$$

$$t_r = 0.0139$$

$$\text{efficiency}(\eta \%) = t_r \times 100 = 1.39$$

### 3.5.3 Drug Delivery

The outermost layer of the skin, the stratum corneum has been considered as the rate limiting barrier to the ionic permeant (Ruddy and Hadzija, 1995). Stratum corneum is composed of dead keratinized cells, embedded in a multicellular lipid matrix which is heterogeneous and lipophilic in nature. Depending upon the physiological properties of the drug, the permeant will follow the pathway of least resistance (Flynn, 1990).

The TRZ flux across skin as a function of time increased linearly with time and reached maximum in about 6 hrs (Figure 3.7). After that, there is steady decrease in flux with time.

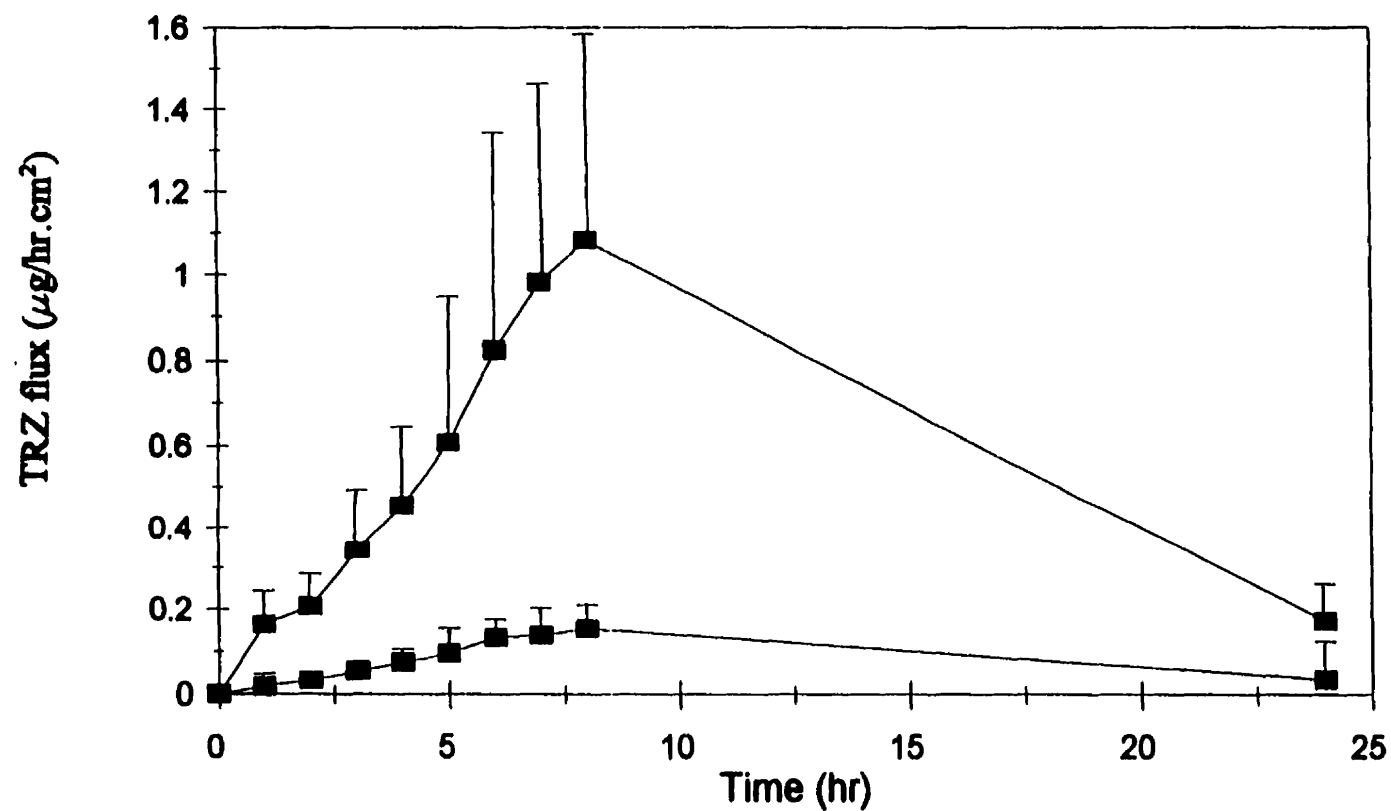
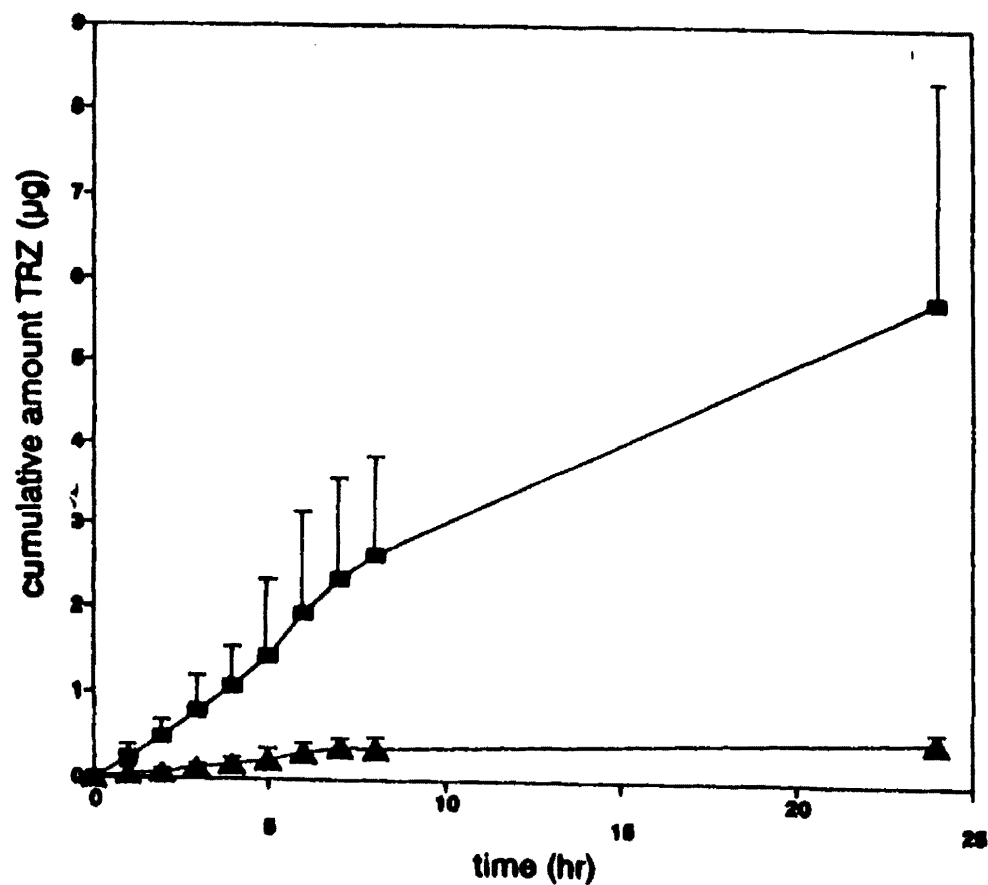


Figure 3.7 TRZ Flux Across Rat Skin after 24 hr delivery. The values shown are mean + SD (n = 5). ■ Iontophoretic delivery ▲ Passive delivery



It was also noted that there is no abnormal increase in drug flux suggesting that current density of  $0.03 \text{ mA/cm}^2$  did not produce any gross skin damage. If the skin was damaged, an abnormal increase in flux would have been noted. Since the drug in reservoir was infinite, the decline in curve could not be easily explained. Further sampling points between 8 to 24 hours probably would give a better estimation of the drug flux. Alternatively, the drug transport could be studied by the plot of cumulative amount of TRZ delivered across skin as a function of time (Figure 3.8). The curve resembled a curve typical of an infinite dose. That is, on increasing drug delivery beyond 24 hours, the amount delivered across skin would be expected to increase linearly till the near depletion of the drug from the reservoir, and the curve would assume a sigmoidal shape. It was also noted that there is no abnormal increase in drug delivery suggesting that the current density of  $0.03 \text{ mA/cm}^2$  did not produce any gross skin damage. If the skin was damaged, an abnormal increase in delivery would have been noted. From the x axis intercept of the linear portion of the plot the lag time required to reach the steady state was obtained. The lag time was negligibly small ( $\sim 2 \text{ min}$ ) suggesting that iontophoresis can rapidly overcome the rate limiting barrier, the stratum corneum. In iontophoresis, the lag time is generally very short. These very short lag



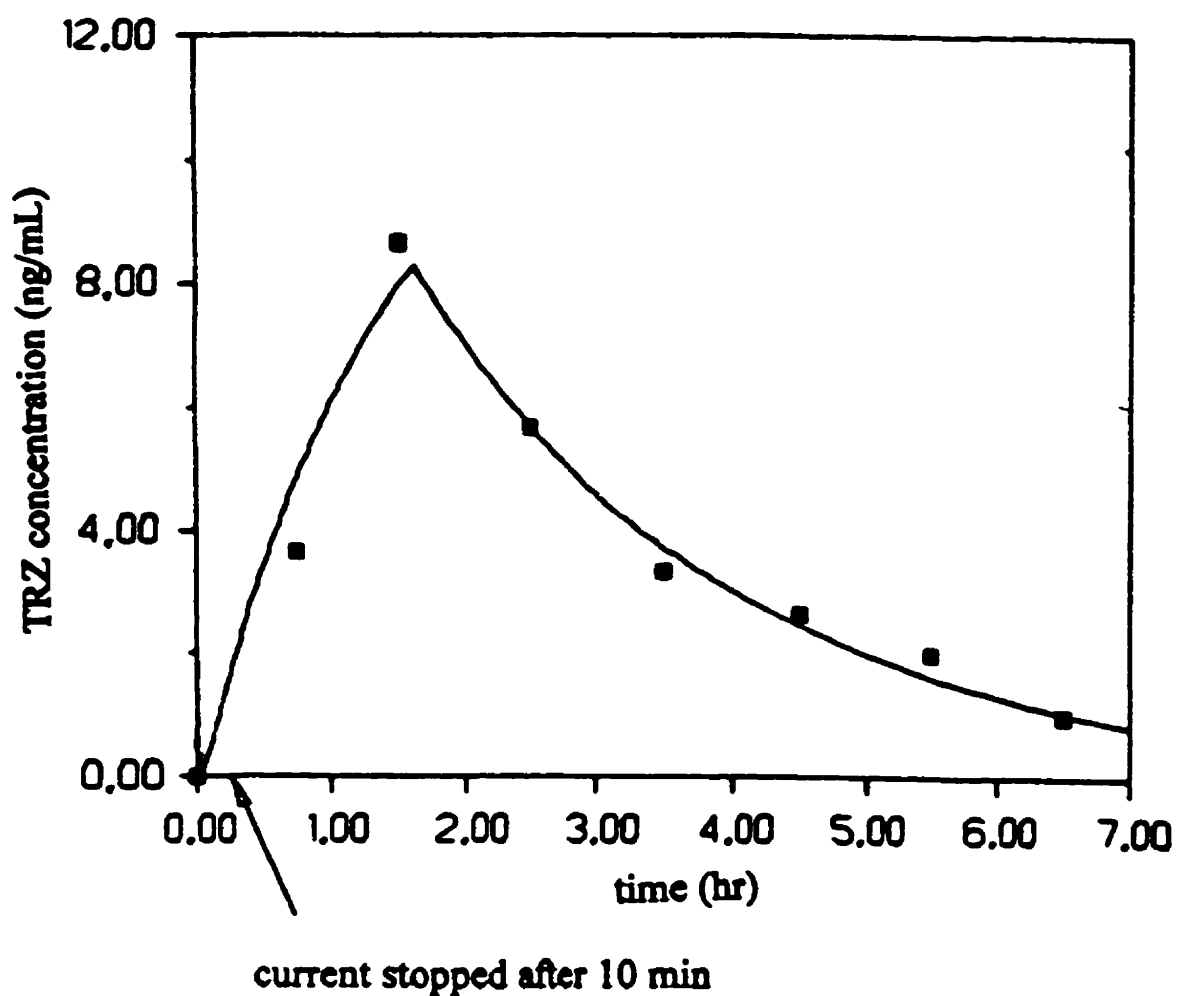
**Figure 3.8 Cumulative Delivery of TRZ Across Excised Rat Skin.** The values shown are mean  $\pm$  SD. Iontophoresis was done in diffusion cells for 24 hrs using: Pt/Pt electrodes and 0.25 mA DC reversing current. ■ Iontophoretic delivery    ▲ Passive delivery

time have raised questions about the nature of the barrier which could be breached very quickly (Singh and Roberts, 1993). This led to several *in vivo* experiments (Haak and Gupta, 1994; Meyer et al., 1989). In these experiments the correlation of drug concentration profile by TI and other parenteral routes suggest that electrically assisted absorption is rapid and the pharmacokinetics is similar to the parenteral route (Haak and Gupta, 1994; Meyer et al., 1989). It is believed that the electric field propels the ionic molecule down its transport pathway and its motion being less impeded because it cannot partition into the abundant lipid moiety along its route. After the passage through the stratum corneum, the drug may accumulate in the dermis where they are absorbed into the blood circulation, and the rate of drug absorption depends upon the dermal blood perfusion (Singh et al., 1994).

As evident from our results (Figure 3.8, Table 3.3), the plasma level of TRZ beyond the delivery time is unlike the parenteral delivery which is always associated with immediate decline in blood levels following termination of delivery. The initial drug release rate exceeds the elimination rate till about 1.5 hr. Thereafter, the elimination rate exceeds the absorption rate. This lends support to the establishment of a

reservoir (Siddiqui and Chein, 1987; Kari et al., 1986) in the skin. Comparing the slopes of the elimination phases of TRZ after TI ( $k_e = 0.308 \text{ hr}^{-1}$ ; Figure 3.9) and that obtained by oral absorption ( $k_e = 0.301 \text{ hr}^{-1}$ ; cr. Chang et al., 1984) in rats suggest that the elimination phase of the two is superimposable.

Skin environment could also influence drug absorption. There is a possibility of drug delivery in the glands (Gibson 1974). The pH of the gland pore on skin is about 4-5, while in dermis it is 7.0. The change in pH may affect the terazosin solubility ( $pK_a$  7.1) making it more lipid soluble (Burnette and Ongipiattanakul, 1987; Siddiqui et al., 1987). In such cases, movement of the drug across the sweat gland would be the rate limiting step. In experiments conducted by Riviere et al. (1991) co-administration of vasodilator or a vasoconstrictor correspondingly increased and decreased plasma concentration of lidocaine by its direct on the large blood vessels. Terazosin is a peripheral vasodilator (Kyncl, 1986) and iontophoresis as such produces vasodilation in the capillaries. (The clinical example for the latter effect is the erythema observed after the iontophoresis of tap water (Holzle and Alberti, 1987). Considering these effects and the short duration of delivery, rapid enormous delivery exceeding



**Figure 3.9** TRZ Plasma-Time-Concentration Simulation in Sprague Dawley Rats ( $n = 5$ ). The values shown are the mean values. The curve is the best fit line obtained from computer simulation.

Table-3.3 TRZ Plasma-Time- Concentration Profile after a 10 min Iontophoretic and Passive Delivery in Rats

BLOOD										
SAMPLING										
TIME	CONCENTRATION OF					TRZ (ng/mL)		spd-P1	spd-P2*	mean
	spd-I1*	spd-I2	spd-I3	spd-I4	spd-I5	mean spd	spd frI1*			spd P
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.75	4.68	4.18	3.22	1.98	4.17	3.65	3.75	0.00	0.00	0.00
1.5	5.46	14.86	9.00	27.42	6.58	8.66	9.91	0.00	0.57	0.30
2.5	10.70	5.00	4.74	5.37	2.60	5.68	20.20	0.00	0.04	0.01
3.5	6.20	2.65	4.21	1.67	1.02	3.34	2.13	0.00	0.08	0.04
4.5	4.79	2.25	3.22	1.26	1.60	2.62	1.51	0.00	0.07	0.04
5.5	2.46	2.04	2.98	1.09	1.36	1.99	0.37	0.00	0.07	0.04
6.5	0.00	0.64	2.53	1.00	0.89	1.00	0.00	0.00	0.03	0.01

\*

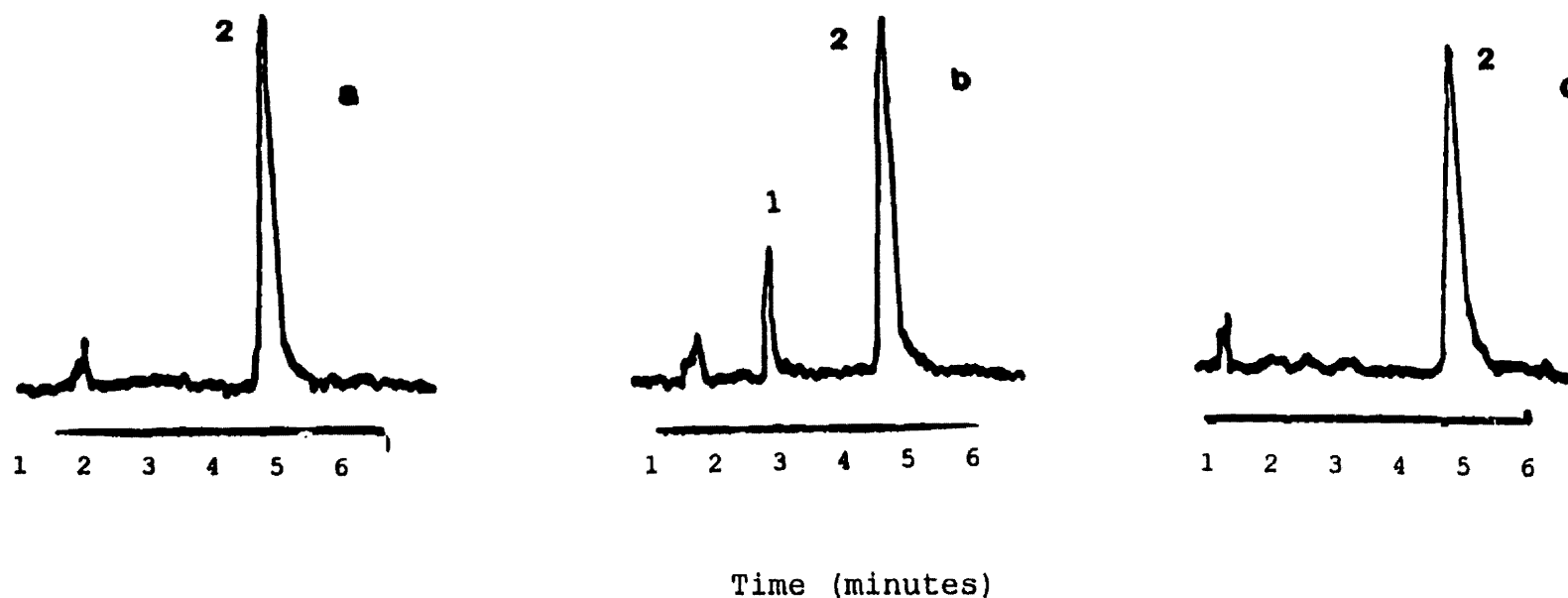
spd-I1 = The suffix I1 refers to iontophoretic delivery in Sprague Dawley rat No 1.  
fr = Fuzzy rat; spd P2 refers to passive delivery in Sprague Dawley rat No 2.

the vascular uptake is unlikely. If this is not true, then some of the drug if not all must appear immediately in blood. This was not evident at 20 min post drug delivery. Several explanation can be forwarded to explain this effect. It is possible that the drug is delivered in apocrine gland, where the blood perfusion is unable to extract the drug. The evidence comes from the clinical evaluation of dermatological disorder of miliaria (Hirksman et al., 1985) where the eccrine gland (analogous comparison as rat do not have eccrine gland over its general skin surface) closes its aperture due to occlusion, and the gland wall being non leaky causes the accumulation of sweat in the duct. The delivery of anticholinergic drug pilocarpine in the diagnosis of cystic fibrosis is itself a suggestion of electric current traveling down the eccrine gland (Gibson, 1975). High current strength and increasing hydration may result in the closure of the shunt route as seen in some cases (Holzle and Alberti, 1987; Millard and Barry, 1988). In such instances the paracellular transport through the lipid bi-layer region would be the predominant pathway in the absence of low resistant pores (Lee et al., 1996).

The pilomotion and the vasoconstrictor responses seen after 30 sec iontophoresis of phenylephrine support the above

belief (Hörniquet et al., 1984). The pilomotion effect was seen only after the vasoconstrictor response and usually at higher current strength. But both responses persisted beyond 30 mins which may not be due to depot effect. Rather the drug's intrinsic affinity at its receptor may play an important role. (The pilomotion and the vasoconstrictor response are mediated by  $\alpha$  receptors.) Jodoul et al. (1995) have shown that the amount of ionic drug in the skin is the same for different duration of drug delivery, suggesting that the initial amount of drug delivered was sequestered at the skin binding sites. Once the binding sites are saturated it is possible that the excess drug is then available for the systemic uptake.





**Figure 3.10 TRZ and PRZ HPLC Chromatograms After a 10 minutes Iontophoresis of TRZ (*in vivo* study).** Chromatogram (a) is from a blank plasma sample. Chromatogram (b) is from the sample collected at 1.5 hr post iontophoresis and (c) is from the plasma collected at 1.5 hour post passive delivery. All samples were spiked with the internal standard (PRZ). The retention times of standard TRZ and PRZ are  $3.1 \pm 0.2$  and  $5.6 \pm 0.4$  minutes respectively.

1 is TRZ peak, 2 is PRZ peak

### 3.6 Summary of Iontophoretic Study

The results from drug delivery study suggest that (1) the delivery of terazosin into the skin is enhanced by iontophoresis in comparison to passive delivery (Table 3.3), and the use of salt-bridge-platinum electrodes prevented terazosin degradation in the presence of electric current. (2) A depot formation of terazosin is seen *in vivo* after 10 minute iontophoretic delivery in rats. The site of depot formation is unknown.

#### 4.0 General Discussion

The analytical procedure developed for terazosin was sensitive, precise, and was employed in the solid phase extraction study. The recovery of terazosin from rat plasma using cyanopropyl columns is high and it is possible to improve the extraction efficiency of the reused columns by altering the column wash cycle.

The analytical procedure developed was applied for terazosin but not for the isolation and characterization of its drug metabolites. The single dose pharmacokinetic study was undertaken to demonstrate the applicability of the analytical procedure rather than a detailed study.

The experiments from the *in vivo* delivery suggest that it is possible to take advantage of a drug reservoir effect in skin to establish intradermal depot of therapeutically useful compounds, which are then slowly absorbed over prolong period. A similar depot effect was seen *in vivo* with the iontophoresis of bretylium tosylate (MW 414) (Kellog et al., 1989), which selectively abolished the vasoconstrictor response in a small skin area without eliciting systemic responses. A prolonged effect may reduce the frequency of drug administration. However, further experiments may be required to validate the reservoir effect. For example, the simulation of intradermal

skin depot as in the iontophoresis of terazosin by the subcutaneous injection may shed more light, but the site of depot formation by the two processes may differ from one another. A mathematical determination of absorption rate constants could provide an alternate and a better estimate.

To improve the patient compliance, the applied current density, the area of application, the pH and the ionic strength of the terazosin solution, and the current amplitude and frequency of DC reversing current can be optimized to produce a maximum delivery with minimum side effects (Huang et al., 1996a; 1996b) such as tingling, itching, slight pricking and erythema. Skin resistance which is highly variable depend upon factors such as degree of hydration, hair and sweat gland density, which can lead to high current density across pores than at the skin surface resulting in current induced skin damage. This problem is avoided by increasing current strength gradually from zero to the desired strength and then maintaining it irrespective of changes brought in the skin impedance. That is, a constant current strength rather than constant voltage will be used. By empirical determinations a current density of  $0.5 \text{ mA}/10 \text{ cm}^2$  can be easily applied to the skin (Sanderson et al., 1989). Since temporary skin changes accompany all iontophoretic

procedures, an optimum time period would be required before a second dose is administered at the same site, or new skin sites may be selected.

Delivery of a drug at the site of action with minimum side effects is one of the advantages of iontophoresis over oral delivery. Unlike the problems associated with an oral route, iontophoresis is independent of gastrointestinal emptying rate and the amount of a drug delivered is easily controlled by varying the current strength or the time of delivery, thus reducing systemic toxic effect. Iontophoresis reduces intra- subject variation (similar drug fluxes were obtained across human thigh skin varying in skin hair density (Burnette and Ongipattanakul, 1987) and inter species variation (comparable fluxes were noted in hairless rats, fuzzy rats, human, pig and rabbit skin (Siddiqui and Chien, 1987; Phipps et al., 1989)).

Initial attempts in our laboratory to develop the Kim and Chung (1992) L<sub>5</sub>/L<sub>6</sub> spinal nerve ligation rat model of causalgia have been unsuccessful. It appears that the age of the rat (Chung et al., 1995) and tightness of the ligature are critical for the animal's robust behavior (allodynia, hyperalgesia) that is the characteristic of causalgia. Presently, no animal models that is truly representative of

the human condition exist, and therefore, further experimental work is necessary to perfect the model. The safety, efficacy and toxicity study in a rat model and in at least three other animal species would be carried out before a phase I human study is initiated.

The fall in blood pressure after delivery may be monitored and could be used as an index to optimize the dose as to achieve a local therapeutic action. A dramatic fall in blood pressure will indicate that smaller iontophoretic dose is required which could be obtained either by decreasing current strength or the time of delivery or both. Once the dose is predetermined, the dose can be programmed to the patient's need.

The size of a device could be reduced to that of a wristwatch, which could then be easily strapped distally to the site of injury. Any error that arises in the working of the iontophoretic system can be indicated by introducing electronic features. But the cost of electrodes and batteries required to sustain the circuit system may make the device expensive. One way of compensating cost is to improve the efficiency of the delivery system. Extraneous ions that are introduced into the drug reservoir compete with drug ions for transport resulting in decreased iontophoretic efficiency. An

efficiency of 1.39% for terazosin, although comparable to compounds of similar molecular weight (Singh et al., 1984) is significantly low, and the needs for better designs are warranted. Penetration enhancers increased the drug flux of large molecular weight compounds, but the combination of iontophoresis and chemical adjuvants was ineffective for low molecular weight compounds (Hirvonen et al., 1993) like terazosin.

The main disadvantage of iontophoresis is that its long term effects of drug and iontophoresis on skin are still to be evaluated. However, the system when used properly can be extremely useful. The administration of lidocaine in the anaesthesia of ear and the iontophoresis of pilocarpine in the diagnosis of cystic fibrosis are generally accepted to be a safe procedure (Echols et al., 1975; Gibon, 1975). As with other novel delivery system, the acceptability of the device depends upon the patient compliance. The management of so far unmanageable pain as in causalgia may enhance the utility value of the device which may outweigh its disadvantages.

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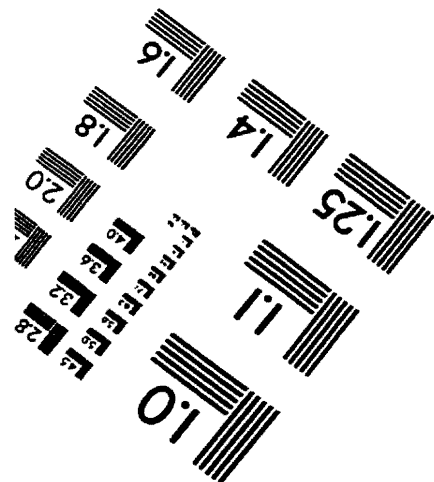
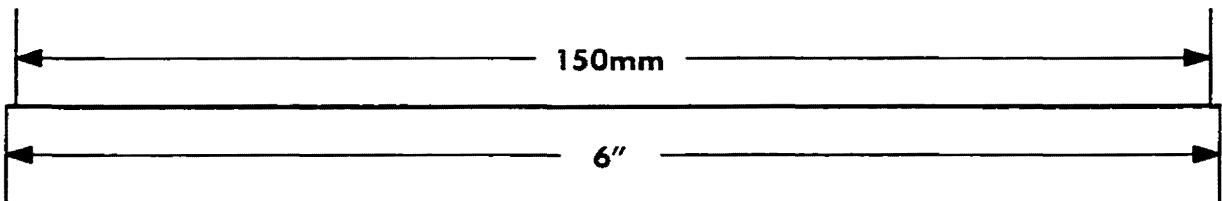
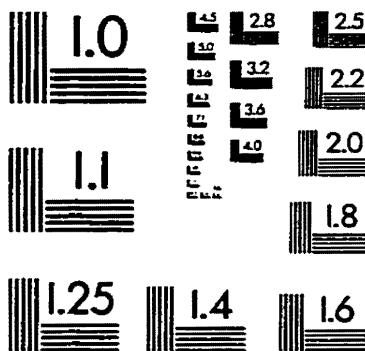
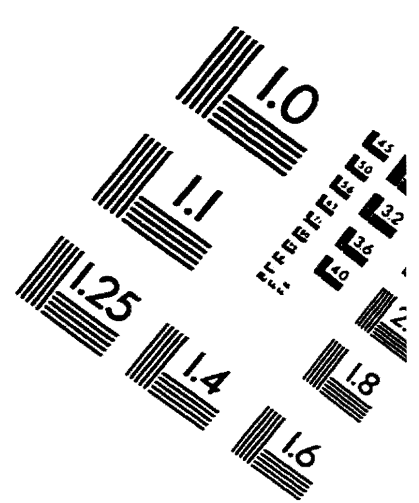
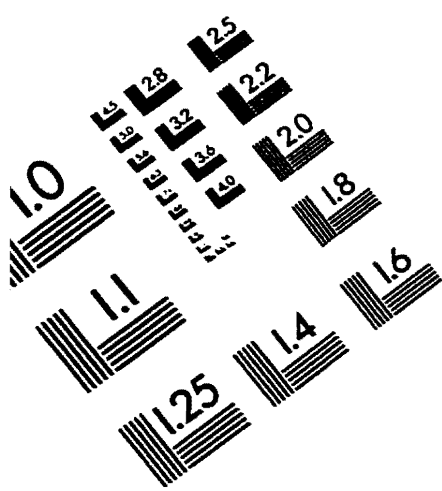
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